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FOR: **SYNTHETIC POLYSACCHARIDES, METHODS FOR
PREPARING SAME AND PHARMACEUTICAL
COMPOSITIONS CONTAINING SAID
POLYSACCHARIDES**

CERTIFICATE UNDER 37 C.F.R. 1.10

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**SYNTHETIC POLYSACCHARIDES, PROCESS FOR THEIR PREPARATION AND
PHARMACEUTICAL COMPOSITIONS CONTAINING THEM**

The present invention relates to novel synthetic polysaccharides having the pharmacological activities of
5 heparin but exerted selectively.

Heparin belongs to the glycosaminoglycan (GAG) family, which are natural heterogeneous sulphated polysaccharides.

The heparin preparations are mixtures of chains comprising a number of monosaccharide units ranging from 10 to
10 100 and more. To this heterogeneity of size is added a heterogeneity of structure, at the level of the nature of the constitutive monosaccharides, but likewise at the level of the substituents which they carry (L. Rodon in: The Biochemistry of Glycoproteins and Glycosaminoglycans, Ed by Lennarz W.J.,
15 Plenum Press, 1980, New York and London, 267-371).

Each natural GAG family in general has a range of pharmacological activities. All are combined in the preparations which can be obtained starting from natural products. Thus, for example, the heparins and the sulphated
20 heparans have an antithrombotic activity which is linked to the simultaneous action on several coagulation factors. They likewise exert an action on a number of growth factors, the most well-known being the basic fibroblast growth factor (bFGF).

25 This action is manifested by an effect on the proliferation of the smooth muscle cells and on angiogenesis. Heparin additionally exerts effects on autoimmunity, inflammation and the formation of tumour metastases.

Heparin especially catalyses the inhibition of two
30 enzymes which intervene in the blood coagulation cascade, namely factor Xa and factor IIa (or thrombin). The heparin preparations of low molecular weight (LMWH) contain chains formed of 4 to 30 monosaccharides and have the property of acting more selectively on factor Xa than on thrombin.

35 Certain synthetic oligosaccharides, especially those described in EP 84999, have the property of selectively inhibiting, via antithrombin III, factor Xa without any activity on thrombin.

The Patent US 5,378,829 describes sulphated glycosaminoglycanoid derivatives of the heparin or heparan sulphate type having an activity which is antithrombotic and inhibitory of the proliferation of smooth muscle cells. This document, however, only describes oligosaccharides having at the maximum 7 monosaccharide units.

In the course of previous years, the tendency in the field of GAGS was to investigate especially oligosaccharides of the lowest molecular weight possible, but it has been subsequently observed that several of the abovementioned biological activities are borne by fragments having at least 8 saccharide units. For example, the inhibitory activity of thrombin would necessitate fragments containing at least between 14 and 20 saccharide units whereas for the activation of bFGF at least 12 saccharide units would be necessary (M. Maccarana et al., J. Biol. Chem., 1993, 268, 23998-23905; J.E. Turnbull et al., J. Biol. Chem., 1992, 267, 10337-10341).

A synthesis of polysaccharides of this size presents great difficulties and, in fact, it has never been carried out.

With the aim of discovering the activity of products of lengthy size, it has been proposed to connect two oligosaccharides of small size by an entity which does not intervene in the biological activity. EP 649854 describes such derivatives having antithrombotic activity. Analogously, WO 95 05182 describes conjugates active on the regulation of bFGF.

These products in fact have a selective activity on the different coagulation factors and inhibitory properties of growth factors expressed by an inhibition of cell proliferation.

It has now been found that fragments of GAGs (glycosaminoglycans) containing 8 or more saccharide units and which can be synthesized relatively simply have biological activities which are not only selective but also quantitatively high.

More particularly, it has been found that it is possible to modulate quantitatively the activity of the said polysaccharides according to the length of the chains and the

distribution of the functional substituents.

Thus, for example, it has surprisingly been found that sulphated and alkylated deca-saccharides can be potent antithrombotics or selective inhibitors of bFGF according to the arrangement of the alkyl groups and of the sulphate groups in the deca-saccharide linkage and that it is likewise possible to obtain selective inhibitors of factor Xa, for example with a tetradeca-saccharide or products having an activity of the heparin type, namely an activity as much on factor Xa as on thrombin, for example with a hexadeca-saccharide.

More generally, it has been found that by production of disaccharide sequences formed by a uronic acid and by a hexose so as to form polysaccharides of 8 to 24 monosaccharide units whose hydroxyl groups are all substituted by alkyl groups or by sulphate groups, it is possible to modulate with precision the activities of the GAGs type to obtain very active and selective products.

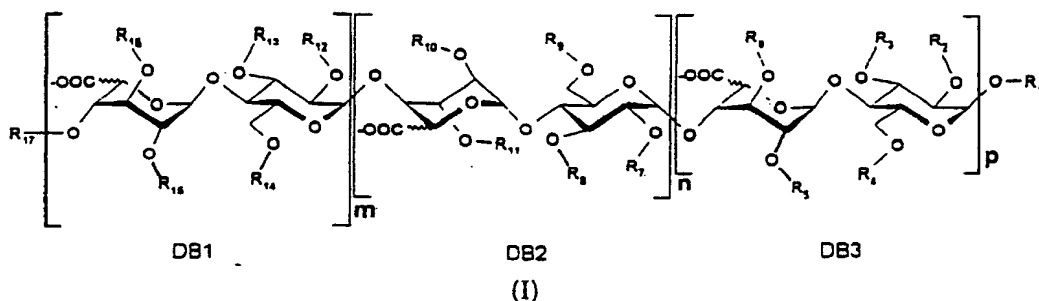
Thus, according to one of its aspects, the present invention relates to a novel synthetic polysaccharide containing from 8 to 24 monosaccharide units formed by a sequence of disaccharides formed from a uronic acid and from a hexose, the said polysaccharide being characterized in that all its hydroxyl groups are etherified with a (C₁-C₆)alkyl group or esterified in the form of a sulfo group, each disaccharide being at least monoetherified; as well as its salts, especially pharmaceutically acceptable salts.

Preferably, the invention relates to a polysaccharide such as defined above, characterized in that all its hydroxyl groups are etherified by a methyl group or esterified in the form of a sulfo group, each disaccharide being at least monoetherified; and to its salts, especially pharmaceutically acceptable salts.

Advantageous products are alkylated, preferably methylated, on the hydroxyls in position 2 and 3 of the uronic acid, the said uronic acid preferably being glucuronic acid or iduronic acid and being trisulphated on the hexose, the said hexose preferably being glucose. Another group of preferred products is formed from polysaccharides which are alkylated,

preferably methylated, on the hydroxyls in position 3 of the uronic acid, the said acid preferably being glucuronic acid or iduronic acid and being alkylated, preferably methylated, on the hydroxyl in position 3 of the glucose.

5 The products of the present invention are thus preferably formed from regular sequences of repeated disaccharides formed in turn of glucuronic acid and of glucose or of iduronic acid and of glucose, represented by the following formula:



in which

- the wavy line indicates either a bond below or above the plane of the pyranose ring;

- R_1 , R_6 , R_{11} and R_{16} are a (C_1-C_6) alkyl;

15 - R_2 , R_3 , R_4 , R_5 , R_7 , R_8 , R_9 , R_{10} , R_{12} , R_{13} , R_{14} , R_{15} and R_{17} are a (C_1-C_6) alkyl or an SO_3^- group;

20 - m , n and p are such that the sum $m + n + p$ is greater than or equal to 4 and less than or equal to 12, one or two of the three being able to be zero, or one of their salts, especially pharmaceutically acceptable salts.

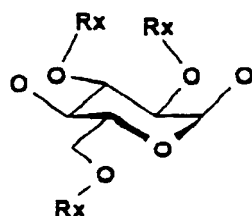
It will be generally noted that in the present invention a wavy line indicates either a bond below or above the plane of the pyranose ring.

25 In the general formula (I) and in the present description, the structures (a) or (b) below represent a glucose skeleton in the ${}^4C_1$ conformation. The structures (c) or (d) below represent a uronic acid skeleton which is either of L-iduronic acid (represented here in its ${}^1C_4$ conformation) or of D-glucuronic acid (represented here in its ${}^4C_1$ conformation).

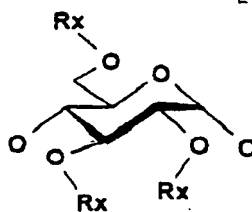
30 On these structures (a), (b), (c) and (d), the R_x

substituents have the definitions attributed for R_1 to R_7 , in (I).

Thus, the structures (a) and (b) are the same representation of a glucose skeleton in the ${}^4C_1$ conformation.

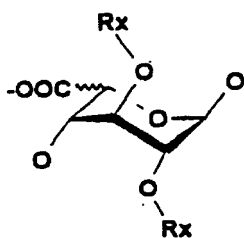


(a)

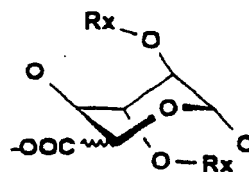


(b)

The structures (c) and (d) represent a uronic acid skeleton which is either of L-iduronic acid (represented here in its ${}^1C_4$ conformation) or of D-glucuronic acid (represented here in its ${}^4C_1$ conformation).



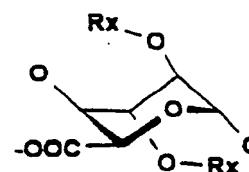
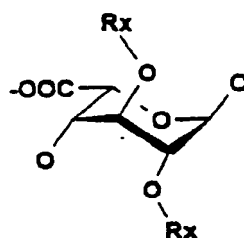
(c)



(d)

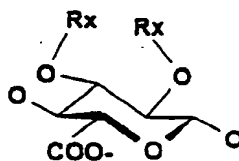
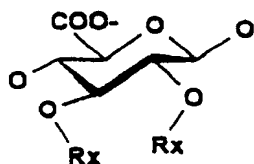
When the structure is L-iduronic acid (c) and (d)

are the following conformers:



When the structure is D-glucuronic acid (c) and (d)

acid (c) and (d) are the following conformers:



In the present description, it has been chosen to

represent the ${}^1C_4$ conformations for L-iduronic acid, ${}^1C_1$ conformations for D-glucuronic acid, but it is well known that the conformation in solution of the monosaccharide units is
10 variable.

The disaccharides DB1, DB2 and DB3 represent identical or different disaccharides.

Preferred compounds according to the invention are those of formula (I) in which n and p are equal to zero and m
15 is 4 to 10 and their salts, especially pharmaceutically acceptable salts.

Equally advantageous are the compounds of formula (I) in which n and p are equal to zero, m is 4 to 10; at least one of the substituents R_{12} , R_{13} , R_{14} and R_{15} is a sulphate group; R_1 ,
20 R_{16} and R_{17} being as defined for (I) as well as their salts, especially pharmaceutically acceptable salts.

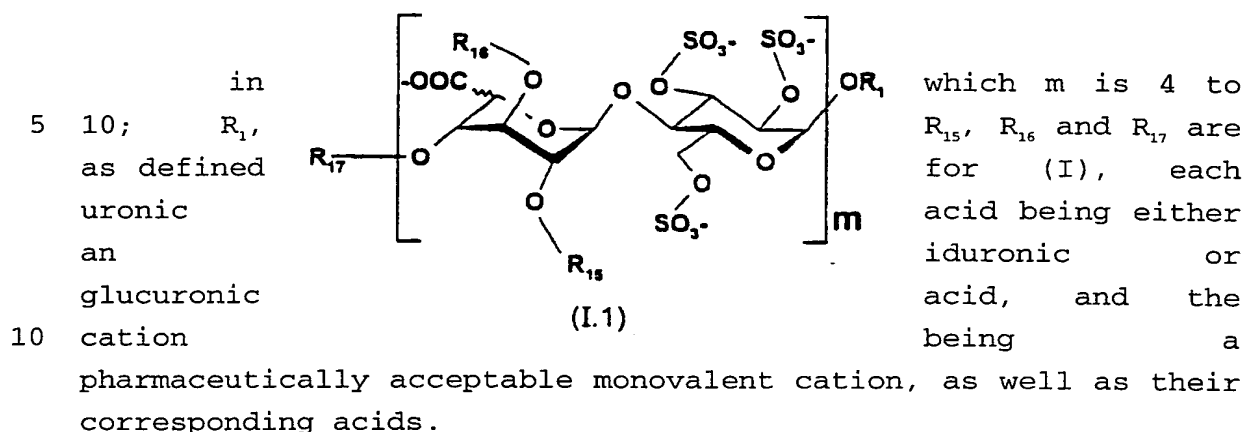
Equally advantageous are the compounds of formula (I) in which n and p are equal to zero, m is 4 to 10; at least two of the substituents R_{12} , R_{13} , R_{14} and R_{15} are a sulphate group; R_1 ,
25 R_{16} and R_{17} being as defined for (I) as well as their salts, especially pharmaceutically acceptable salts.

Equally advantageous are the compounds of formula (I) in which n and p are equal to zero, m is 4 to 10; at least three of the substituents R_{12} , R_{13} , R_{14} and R_{15} are a sulphate
30 group; R_1 , R_{16} and R_{17} being as defined for (I), as well as their salts, especially pharmaceutically acceptable salts.

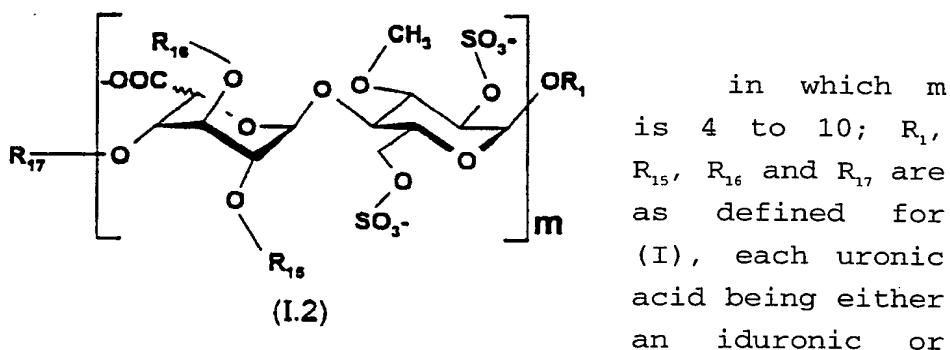
Equally advantageous are the compounds of formula (I) in which n and p are equal to zero, m is 4 to 10; the groups R_{12} , R_{13} , R_{14} and R_{15} all represent a sulphate group; R_1 , R_{16} and R_{17} ,
35 being as defined for (I) and their salts, especially pharmaceutically acceptable salts.

Other advantageous compounds are the salts formed from an anion and from a cation, the anion corresponding to the

formula (I.1):



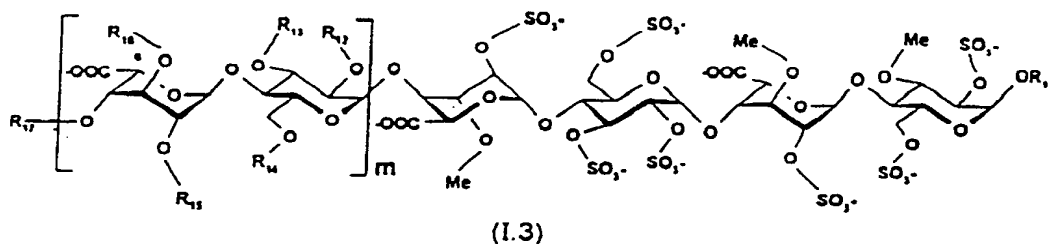
The salts formed from an anion and from a cation where the anion corresponds to the formula (I.2):



25 acceptable monovalent cation, as well as their corresponding acids, are equally advantageous.

In the formulae (I), (I.1), (I.2) above, the etherifying alkyl groups are preferably methyls.

30 The salts formed from an anion and from a cation, where the anion has the formula (I.3):



in which m is 2 or 3, R_1 , R_{12} , R_{13} , R_{14} , R_{15} , R_{16} and R_{17} , being as defined for (I), each uronic acid being either an iduronic or glucuronic acid and where the cation is a pharmaceutically acceptable monovalent cation, as well as their corresponding acids, are particularly advantageous.

Among these compounds (I.3), those are very particularly preferred for which R_1 is a methyl, R_{13} in position 3 of the glucose is a methyl, R_{12} in position 2 and R_{14} in position 6 of the glucose are an SO_3^- and R_{16} in position 3 of the iduronic or glucuronic unit is a methyl, m being equal to 2 or 3.

The preferred salts of the invention are those of which the cation is selected from the cations of the alkali metals and most preferably also those of which the cation is Na^+ or K^+ .

The following polysaccharides are particularly preferred:

1) methyl (1-4)-O-(2,3-di-O-methyl-4-O-sulpho- α -L-idopyranosyluronic acid)-[(1-4)-O-(2,3,6-tri-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl- α -L-idopyranosyluronic acid)]₅-2,3,6-tri-O-sulpho- α -D-glucopyranoside, sodium salt,

2) methyl (1-4)-O-(2,3-di-O-methyl-4-O-sulpho- α -L-idopyranosyluronic acid)-[(1-4)-O-(2,3,6-tri-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl- α -L-idopyranosyluronic acid)]₄-2,3,6-tri-O-sulpho- α -D-glucopyranoside, sodium salt,

3) methyl (1-4)-O-(2,3-di-O-methyl-4-O-sulpho- α -L-idopyranosyluronic acid)-[(1-4)-O-(2,3,6-tri-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl- α -L-idopyranosyluronic acid)]₃-2,3,6-tri-O-sulpho- α -D-glucopyranoside, sodium salt,

4) methyl (1-4)-O-(2,3-di-O-methyl-4-O-sulpho- α -L-idopyranosyluronic acid)-[(1-4)-O-(2,3,6-tri-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl- α -L-idopyranosyluronic acid)]₂-2,3,6-tri-O-sulpho- α -D-glucopyranoside, sodium salt,

5) methyl (1-4)-O-(2,3-di-O-methyl-4-O-sulpho- α -L-idopyranosyluronic acid)-[(1-4)-O-(2,3,6-tri-O-sulpho- α -D-

glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl- α -L-idopyranosyluronic acid)],-2,3,6-tri-O-sulpho- α -D-glucopyranoside, sodium salt,

6) methyl (1-4)-O-(2,3-di-O-methyl-4-O-sulpho- α -L-idopyranosyluronic acid)-[(1-4)-O-(2,3,6-tri-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl- α -L-idopyranosyluronic acid)],-2,3,6-tri-O-sulpho- α -D-glucopyranoside, sodium salt,

7) methyl (1-4)-O-(2,3-di-O-methyl-4-O-sulpho- β -D-glucopyranosyluronic acid)-[(1-4)-O-(2,3,6-tri-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl- β -D-glucopyranosyluronic acid)],-2,3,6-tri-O-sulpho- α -D-glucopyranoside, sodium salt,

8) methyl (1-4)-O-(2,3-di-O-methyl-4-O-sulpho- β -D-glucopyranosyluronic acid)-[(1-4)-O-(2,3,6-tri-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl- β -D-glucopyranosyluronic acid)],-2,3,6-tri-O-sulpho- α -D-glucopyranoside, sodium salt,

9) methyl (1-4)-O-(3-O-methyl-2,4-di-O-sulpho- α -L-idopyranosyluronic acid)-[(1-4)-O-(3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(3-O-methyl-2-O-sulpho- α -L-idopyranosyluronic acid)],-3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranoside, sodium salt,

10) methyl (1-4)-O-(3-O-methyl-2,4-di-O-sulpho- α -L-idopyranosyluronic acid)-[(1-4)-O-(3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(3-O-methyl-2-O-sulpho- α -L-idopyranosyluronic acid)],-3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranoside, sodium salt,

11) methyl (1-4)-O-(3-O-methyl-2,4-di-O-sulpho- α -L-idopyranosyluronic acid)-[(1-4)-O-(3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(3-O-methyl-2-O-sulpho- α -L-idopyranosyluronic acid)],-3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranoside, sodium salt,

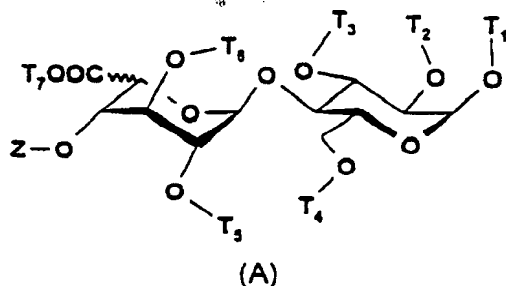
12) methyl (1-4)-O-(3-O-methyl-2,4-di-O-sulpho- β -D-glucopyranosyluronic acid)-[(1-4)-O-(3-O-methyl-2,6-di-O-

13) methyl (1-4)-O-(3-O-methyl-2,4-di-O-sulpho- α -L-idopyranosyluronic acid)-[(1-4)-O-(3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(3-O-methyl-2-O-sulpho- α -L-idopyranosyluronic acid)]₂-(1-4)-O-(2,3,6-tri-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(3-O-methyl-2-O-sulpho- α -L-idopyranosyluronic acid)-3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranoside, sodium salt,

14) methyl (1-4)-O-(3-O-methyl-2,4-di-O-sulpho- α -L-idopyranosyluronic acid)-[(1-4)-O-(3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(3-O-methyl-2-O-sulpho- α -L-idopyranosyluronic acid)],-(1-4)-O-(2,3,6-tri-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(3-O-methyl-2-O-sulpho- α -L-idopyranosyluronic acid)-3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranoside, sodium salt.

The present invention likewise relates to a process for the preparation of the compounds of formula (I) characterized in that:

(a) a glycosidic link donor monosaccharide is coupled to a glycosidic link acceptor monosaccharide according to the classical methods of sugar chemistry to obtain, after the chemical modifications well known to the person skilled in the art, an intermediate saccharide synthon of completely protected disaccharide type of formula (A):



which the identical
T₁, T₂, T₃, T₄, T₅,
substituents are
from the protective
in sugar chemistry
permanent, semi-
temporary

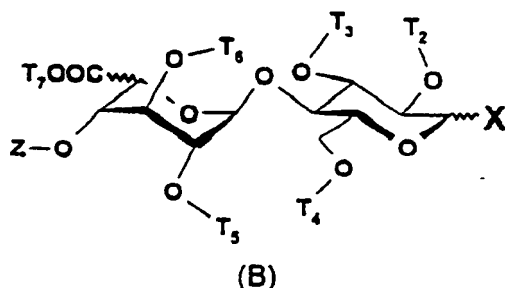
in
or different
T₆, T₇ and Z
selected
groups used
as
permanent or
protective gr

(b) the disaccharide of formula (A) above is modified

chemically so as to obtain an intermediate saccharide synthon of glycosidic link donor disaccharide type of formula (B):

5

in
are such as
(A) and X is
of the
10 such as an



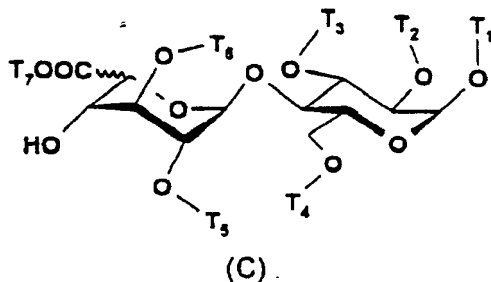
which T_2 to T_5 and Z defined above for an activating group anomeric carbon, imidate, a thioglycoside, a glycoside, a phosphite, a halide

15 pentenyl-xanthate, a or any other group well known for activating the anomeric carbon, then

(c) the disaccharide of formula (A) above is modified chemically so as to obtain an intermediate glycosidic link acceptor saccharide synthon of formula (C):

20

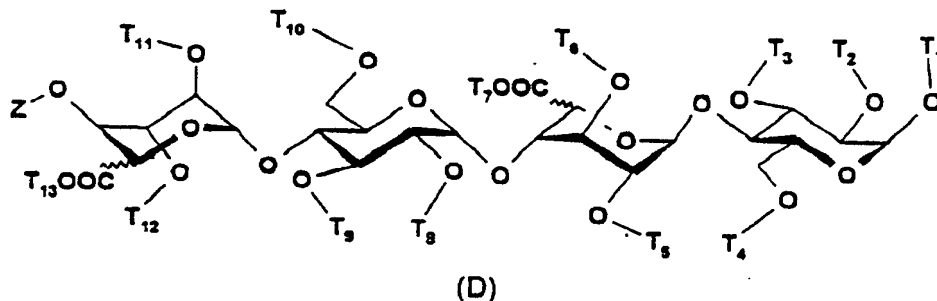
in
such as
selectively
protective
to methods
25 person
then



which T_1 to T_5 are defined for (A), by eliminating the group Z according well known to the skilled in the art,

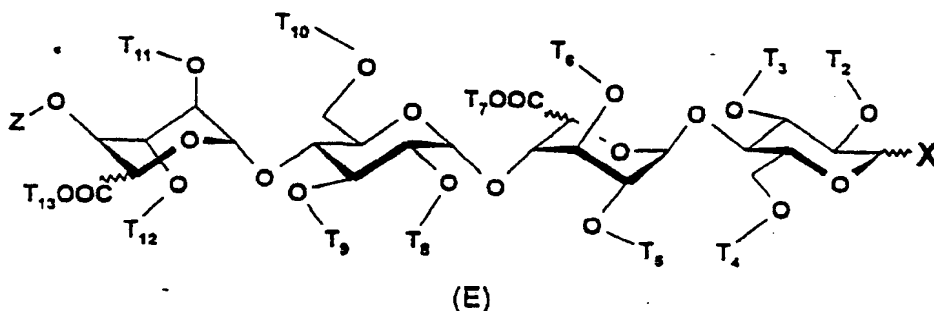
25

(d) a glycosidic link donor disaccharide of formula (B) obtained above and a glycosidic link acceptor disaccharide of formula (C) obtained above are coupled so as to obtain a completely protected tetrasaccharide of formula (D):



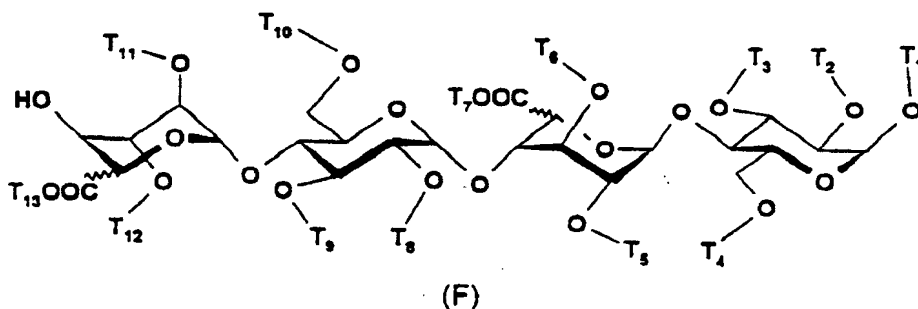
in which T_1 to T_7 and Z are such as defined above for (A) and T_8 , T_9 , T_{10} , T_{11} , T_{12} and T_{13} are such as defined for T_2 to T_7 then,

(e) the tetrasaccharide of formula (D) is then modified
5 chemically so as to obtain an intermediate saccharide synthon of glycosidic link donor tetrasaccharide type of formula (E):



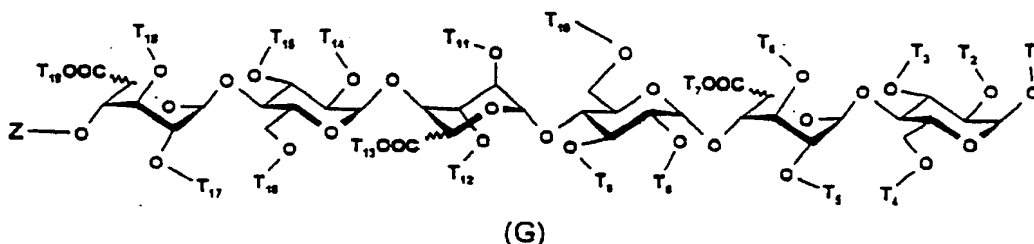
in which X has the same definition as for (B) and T_2 to T_{13} are such as defined for (D) then,

10 (f) the tetrasaccharide of formula (D) is then deprotected selectively so as to obtain a glycosidic link acceptor tetrasaccharide of formula (F):



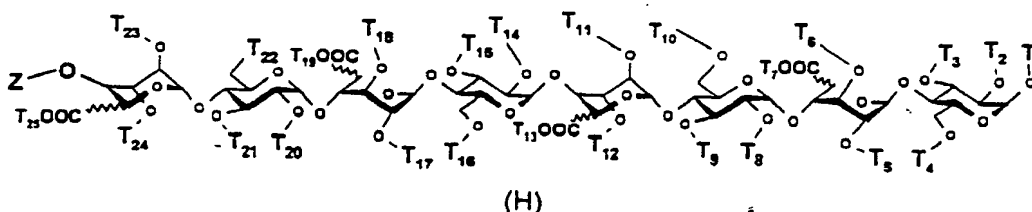
15 in which T_1 to T_{13} are such as defined above for (D) then,

(g) the glycosidic link acceptor tetrasaccharide of formula (F) and a glycosidic link donor disaccharide of formula (B) such as those obtained above are coupled to form an intermediate saccharide synthon of completely protected
20 hexasaccharide type of formula (G):



in which T_1 to T_{13} are such as defined above for (D) and T_{14} to T_{19} are such as defined for T_2 to T_7 for (B),

5 or else the glycosidic link acceptor tetrasaccharide of formula (F) and a glycosidic link donor tetrasaccharide of formula (E) are coupled so as to obtain a completely protected octasaccharide of formula (H):



10 in which T_1 to T_{19} and Z are such as defined previously and T_{20} to T_{25} are such as defined for T_2 to T_7 for (B) then,

(h) the hexasaccharide of formula (G) or the octasaccharide of formula (H) obtained above is modified chemically so as to obtain an intermediate saccharide synthon
15 of glycosidic link acceptor hexasaccharide type of formula (G) in which Z is hydrogen or else a glycosidic link acceptor octasaccharide of formula (H) in which Z is hydrogen,

(i) the above deprotection and coupling steps are repeated until the completely protected oligosaccharide having
20 the desired structure is obtained, the glycosyl donor and glycosyl acceptor intermediate saccharide synthons being chosen as a function of the final structure to thus obtain the protected precursor of the desired final polysaccharide of formula (I), in which the nature of the protective groups T_i
25 determines the position of the alkyl and sulphate groups on the final product (I), and

(j) the deprotection of the alcohol functions which

must be sulphated, and carboxylic acids, is carried out by eliminating the protective groups Ti which protected these functions in the course of the steps of elaboration of the skeleton, then, finally

- 5 (k) the sulphation is carried out to obtain the compounds (I), or one of their salts.

The process described above is the preferred process of the invention. However, the compounds of formula (I) can be prepared by other methods known from sugar chemistry described,
10 for example, in Monosaccharides, their chemistry and their roles in natural products, P.M. Collins and R.J. Ferrier, J. Wiley & Sons, 1995 and in G.J. Boons, Tetrahedron, 1996, 52, 1095-1121.

As a variant, the compounds of formula (I) can be
15 prepared according to the process described in M. Dreef, XVIIth Carbohydrates Symposium, Ottawa, 1-22 July 1994, Abstract D 2.5.

Semi-permanent groups are understood as meaning groups which can first be eliminated after the glycosylation reactions
20 when the carbohydrate skeleton contains the desired number of units, without removal or alteration of the other groups present, allowing, then, the introduction of desired functional groups into the positions which they occupy.

The permanent groups are groups capable of maintaining
25 the protection of -OH radicals during the introduction of functional groups in place of semi-permanent groups.

These groups are selected from those compatible with the functional groups introduced after elimination of the semi-permanent groups. They are, in addition, groups which are inert
30 with respect to reactions carried out for the introduction of these functional groups and which can be eliminated without these functional groups being altered.

According to the invention, the permanent groups are C₁-C₆ alkyl groups.

35 An example of a semi-permanent and/or temporary group which may be mentioned is benzyl and acetate groups.

The substituents in position 3 of the uronic units of the target compound can be already present in the starting

synthons of formula (A), as well as the substituent R_1 .

In the process above, the substituents T_1 , T_6 , T_{12} , T_{18} and T_{24} have the same definition as R_1 , R_6 , R_{11} and R_{16} in the formula (I), that is to say they are a (C_1-C_6) alkyl.

5 The protective groups used in the preparation process of the compounds (I) are those currently used in sugar chemistry, for example, in Protective Groups in Organic Synthesis, TW Greene, John Wiley & Sons, New York, 1981.

10 The protective groups are advantageously chosen, for example, from amongst the acetyl, halomethyl, benzoyl, levulinyl, benzyl, substituted benzyl, optionally substituted trityl, tetrahydropyranyl, allyl, pentenyl, tert-butyldimethylsilyl (tBDMS) or trimethylsilylethyl (...) groups.

15 The activator groups are those classically used in sugar chemistry according to, for example, G.J. Boons, Tetrahedron, 1996, 52, 1095-1121. These activator groups are chosen, for example, from amongst the imidates, the thioglycosides, the pentenylglycosides, the xanthates, the phosphites or the halides.

20 The process described above allows the compounds of the invention to be obtained in the form of salts. To obtain the corresponding acids, the compounds of the invention in the form of salts are contacted with a cation exchange resin in acid form.

25 The compounds of the invention in the form of acids can then be neutralized by a base to obtain a desired salt.

30 For the preparation of the salts of the compounds of formula (I), it is possible to use any inorganic or organic base giving pharmaceutically acceptable salts with the compounds of formula (I).

The base preferentially used is sodium hydroxide, potassium hydroxide, calcium hydroxide or magnesium hydroxide. The sodium and calcium salts of the compounds of formula (I) are the preferred salts.

35 In step (a) of the process, the protective groups used are those usually used by the person skilled in the art in sugar chemistry, for example according to EP 084999 or alternatively according to Protective Groups in Organic

Synthesis, TW Greene, J.Wiley & Sons, 1995.

The protective groups Z, protecting the position 4 of the non-reducing terminal end, and T₁, protecting the position 1 of the reducing terminal unit, can be eliminated selectively to allow the functionalization of the corresponding positions of the disaccharide in the course of the following steps of the synthesis. The nature of the other protective groups T₂ to T₈ is chosen taking account of the alcohol functions which have to be sulphated in the final product. A position carrying an alkyl group in the final product is protected by this group from the start of the synthesis, whereas a position which has to be sulphated is protected by a temporary protective group such as aralkyl (benzyl or substituted benzyl), esters (acetates, benzoates). The position and the nature of the substituents T_i in the final product thus determine the profile of sulphation of the disaccharide fragment originating from (A). It is possible to obtain a great variety of disaccharides of the type of those of formula (A). The preparation of the disaccharides of formula (A) is essentially carried out as described in the Application EP 90201006 or alternatively in the publication C.A.A. van Boeckel and M. Petitou, Angew. Chem. Int. Ed. Engl., 1993, 32, 1671-1690 or alternatively according to G.J. Boons, Tetrahedron, cited above.

According to step (b), it is possible, when T₁ is an alkyl, to use an acetolysis reaction in acetic anhydride, which then serves as a solvent and reagent, in the presence of a strong acid such as sulphuric acid or trifluoroacetic acid to liberate selectively the protective group of the anomeric position. When T₁ is an ester it is possible selectively to eliminate this ester using, for example, benzylamine and an aprotic solvent such as diethyl ether or dichloromethane, or else alternatively N-methylmorpholine in tetrahydrofuran. When T₁ is an allyl group it is possible to isomerize it to vinyl and to then proceed to a mild acid hydrolysis of the vinyl ether, for example in the presence of mercuric chloride in an acetone solution. By means of the methods above, a hydroxylated compound is obtained which is then converted into compound (B), for example by means of a reaction with trichloroacetonitrile

in the presence of a base to obtain in this case a compound (B) in which X is an imidate. In the case where X is a thioglycoside, or a pentenylglycoside, the group T₁ in the compound (A) can be equal to X.

5 According to step (c), it is possible to obtain a glycosidic link acceptor disaccharide by selectively eliminating the group Z according to a process well known depending on the nature of Z, employing, for example, (1) hydrazine acetate or hydrazine hydrate, in pyridine or in
10 toluene, in the case where Z is a levulinic group or else (2) thiourea in ethanol in the case where Z is a trichloroacetyl group.

 According to step (d), a glycosidic link donor disaccharide of formula (B) obtained above and a glycosidic
15 link acceptor disaccharide of formula (C) obtained above are coupled so as to obtain a completely protected tetrasaccharide of formula (D), for this a known activator of the glycosyl donor X is used, for example trimethylsilyl triflate or TMS or *tert*-butyldimethylsilyl triflate or TBDMS or alternatively
20 boron trifluoride in diethyl ether, for example when an imidate is involved, or else the N-iodosuccinimide system or NIS, triflic acid when a thioglycoside is involved or any other system known for activating X according to the references cited above. The solvents used for the reaction are preferably
25 dichloromethane, dichloroethane, chloroform, chlorobenzene, diethyl ether, toluene, benzene; the reaction is carried out under anhydrous conditions, and generally at low temperature between -70 and 0°C. It is also possible to carry out the reaction at ambient temperature.

30 For step (e), the procedure is essentially as described in step (b), starting from the tetrasaccharides obtained according to step (d).

 For step (f), the procedure is as described in (c) to selectively deprotect the tetrasaccharides obtained according
35 to step (d), and the tetrasaccharides of formula (F) are obtained.

 For step (g), the procedure is as described in step (d), advantageously the products of the reaction of formula (G)

are separated by means of gel permeation chromatography.

For step (h), the procedure is essentially as described in step (b), starting from hexasaccharides or from octasaccharides of formula (H) obtained according to step (g).

5 The above deprotection and coupling steps are repeated until the completely protected oligosaccharide having the desired structure is obtained, the glycosyl donor and glycosyl acceptor intermediate saccharide synthons being chosen as a function of the final structure to thus obtain the protected
10 precursor of the desired final polysaccharide of formula (I), in which the nature of the protective groups Ti determines the position of the sulphate and alkyl groups on the final product (I).

15 The protective groups of the alcohol functions which must be sulphated are then eliminated according to step (j), by saponification with sodium hydroxide or lithium hydroxide and/or by catalytic hydrogenation, for example in the presence of palladium on carbon.

20 According to step (k), the sulphation is carried out with the aid of a sulphating agent such as an SO₃-amine complex, for example the pyridine-SO₃ complex, or with the aid of chlorosulphonic acid in an aprotic solvent such as dimethylformamide, preferably at a temperature of between 0°C and 100°C, to obtain the compounds (I) which are optionally
25 purified by gel permeation chromatography using water or a solution of sodium chloride as eluent.

The compounds (I) thus obtained can optionally be salified.

30 The compounds of the formula (I) above likewise comprise those in which one or more hydrogen or carbon atoms have been replaced by their radioactive isotope, for example tritium or carbon 14. Such labelled compounds are useful in research, metabolism or pharmacokinetic work, and in biochemical assays as ligands.

35 The compounds according to the invention have been the subject of biochemical and pharmacological studies which have shown that they have very interesting properties.

The compounds of the present invention which are bound

to AT III ($K_b \leq 200$ nM) or to heparin co-factor (HC II) with an affinity equal to or greater than that of heparin have the anticoagulant properties of heparin.

They therefore inhibit several coagulation factors such
5 as factor Xa (titre $\leq 10 \mu$ of anti-Xa/mg) or thrombin ($IC_{50} \leq 10 \mu$ g/ml) and at this titre are excellent antithrombotic agents in models of venous thrombosis and arterial thrombosis.

The affinity of the compounds of formula (I) for AT III
10 has been determined by spectrofluorometry under the conditions described by D. Atha et al. in Biochemistry, 1987, 26, 6454-6461. The results of the assays have demonstrated that the compounds of the invention have a very high affinity for AT III (K_b of between 0.1μ M and 1 nM).

The anti-factor Xa (anti-Xa) activity of the products
15 of the invention has been evaluated at pH 8.4 according to the method described by Teien A.N. and Lie M., in Thrombosis Research, 1977, 10, 399-410, and it has been demonstrated that some compounds of the invention have an anti-Xa activity equal to or greater than that of the synthetic heparinoids already
20 known, and especially is greater than 50 u of anti-Xa/mg.

As has been mentioned above, in the coagulation cascade factor Xa activates prothrombin to thrombin, which proteolyses soluble fibrinogen with liberation of insoluble fibrin, the principal constituent of the blood clot. The inhibition of
25 factor Xa is thus a favoured means of obtaining an anticoagulant and antithrombotic activity.

The global antithrombotic activity of the products of formula (I) has been evaluated by the intravenous route or subcutaneously in the rat, by a model of venous stasis and
30 induction by thromboplastin, according to the method described by J. Reyers et al. in Thrombosis Research, 1980, 18, 669-674. The ED_{50} of the compounds of the invention is at least of the same order or less than that of the other synthetic heparinoids already known (ED_{50} of between 5 and 500 μ g/kg). The compounds
35 of the invention thus have a specificity of action and a particularly interesting anticoagulant and antithrombotic activity.

The compounds of the present invention which are

capable of modulating, especially of inhibiting, the activity of growth factors, in particular of bFGF, allow the proliferation of vascular smooth muscle cells in culture (CML) to be inhibited with an inhibitory effect which is slightly greater than that of heparin on the growth of the smooth muscle cells.

The action of the compounds of the invention on the activity of bFGF has been evaluated by immobilization of iodinated FGF on human CML in culture.

10 The inhibition of the proliferation of CML has been evaluated *in vitro* on cultures of aortic human CML (R. Ross, J. Cell. Biol., 1971, 172-186).

The compounds of the present invention which also have antiviral or hypolipidaemic properties, anti-free radical properties by liberation of superoxide dismutase, or antimetastatic, antiangiogenic or anti-inflammatory properties, can likewise act on the growth and the differentiation of neuronal cells in culture. They are therefore useful in all the pathologies where perturbations of these biological mechanisms intervene.

20 Some compounds of the invention also exert a protective and regenerative action on the nerve fibres.

Owing to their selective biochemical and pharmaceutical activity, the compounds of the present invention are very interesting medicines. Their toxicity is perfectly compatible with this use. They are also very stable and are thus particularly appropriate for forming the active principle of pharmaceutical specialities.

30 For their activity on coagulation factors, the compounds of the invention can be used in various pathologies connected with coagulation and in particular in disorders of the cardiovascular and cerebrovascular system. More particularly, they have a great affinity for antithrombin III as well as a significant anti-factor Xa and antithrombin activity. The inhibition of these coagulation factors is thus a favoured means of obtaining an anticoagulant and antithrombotic activity.

They can be used in various pathologies following a

modification of the haemostasis of the coagulation system appearing in particular during disorders of the cardiovascular and cerebrovascular system as well as thromboembolic disorders associated with atherosclerosis and with diabetes such as
5 unstable angina, cerebral attack, restenosis after angioplasty, endarterectomy, the fitting of endovascular prostheses; or thromboembolic disorders associated with rethrombosis after thrombolysis, with infarct, with dementia of ischaemic origin, with peripheral arterial diseases, with haemodialysis, with
10 auricular fibrillations or alternatively during the use of vascular prostheses of aorto-coronary by-passes. These products can additionally be used for the treatment or the prevention of thromboembolic pathologies of venous origin such as pulmonary embolisms. They can be used either to prevent or to treat
15 thrombotic complications appearing during surgical interventions or together with other pathologies such as cancer, or bacterial or viral infections. In the case of their use during the fitting of prostheses, the compounds of the present invention can coat prostheses and render them
20 haemocompatible. In particular, they can be attached to intravascular prostheses (stents). In this case, they can optionally be modified chemically by introduction at the non-reducing or reducing end of an appropriate arm, as described according to EP 649 854.

25 Use in restenosis after angioplasty is favoured by the inhibitory properties of certain growth factors, such as bFGF.

The compounds of the present invention can likewise be used as an adjuvant during endarterectomy carried out with porous balloons.

30 The results obtained during different pharmacokinetic studies carried out with the products of the invention have demonstrated that they are very well absorbed by the gastrointestinal tract and that their half-life is long. This allows the possibility of a single daily administration to be
35 envisaged during their use in therapeutics.

These studies have also demonstrated that the pharmaceutical compositions prepared with the products of formula (I), the subject of the present invention, are absorbed

by the digestive tract without the quantities administered being prohibitive for use in human therapeutics. The compounds of the invention are thus useful for the preparation of pharmaceutical compositions, which can be administered just as easily by the parenteral route as by the oral route.

The compounds of the invention are very stable and are thus particularly appropriate for forming the active principle of medicines.

According to another of its aspects, the present invention relates to a pharmaceutical composition containing, as active principle, a synthetic polysaccharide containing from 8 to 24 monosaccharide units formed by a sequence of disaccharides formed from a uronic acid and from a hexose, the said polysaccharide being characterized in that all its hydroxyl groups are etherified with a (C₁-C₆)alkyl group or esterified in the form of the sulpho group, each disaccharide being at least monoetherified; or one of its pharmaceutically acceptable salts. The said synthetic polysaccharide, the active principle of the compositions of the present invention, is preferably alkylated by a methyl group.

The invention preferably relates to pharmaceutical compositions containing, as active principle, a compound of formula (I), (I.1), (I.2), (I.3) or one of its pharmaceutically acceptable salts, optionally in combination with one or more inert and appropriate excipients.

In each dose unit the active principle is present in the quantities adapted to the daily doses envisaged. In general, each dose unit is suitably adjusted according to the dose and the type of administration intended, for example compressed tablets, gelatin capsules and the like, sachets, ampoules, syrups and the like, drops, a transdermal or transmucosal patch so that such a dose unit contains from 0.1 to 100 mg of active principle, preferably 0.5 to 50 mg.

The compounds according to the invention can likewise be used in combination with another active principle useful for the desired therapy such as, for example, antithrombotics, anticoagulants, platelet aggregation inhibitors such as, for example, dipyridamole, aspirin, ticlopidine, clopidogrel or

antagonists of the glycoprotein IIb/IIIa complex.

The pharmaceutical compositions are formulated for administration to mammals, including man, for the treatment of the abovementioned illnesses.

5 The pharmaceutical compositions thus obtained are advantageously presented in various forms, such as, for example, injectable or drinkable solutions, coated tablets, compressed tablets or gelatin capsules. The injectable solutions are the preferred pharmaceutical forms. The
10 pharmaceutical compositions of the present invention are especially useful for the preventive or curative treatment of disorders of the vascular wall, such as atherosclerosis, hypercoagulability states observed, for example, following surgical operations of tumour developments or of coagulation
15 disorders, induced by bacterial, viral or enzymatic activators. The dosage can vary widely as a function of the age, weight and state of health of the patient, of the nature and of the severity of the disorder, as well as of the route of administration. This dosage comprises the administration of one
20 or more doses of approximately 0.1 mg to 100 mg per day, preferably approximately 0.5 to 50 mg per day, by the intramuscular or subcutaneous route, in batchwise administrations or at regular intervals.

 The present invention thus likewise relates to the
25 pharmaceutical compositions which contain as active principle one of the above compounds, optionally in combination with another active principle. These compositions are prepared so that they can be administered by the digestive or parenteral route.

30 In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, transmucosal, local or rectal administration, the active ingredient can be administered in unitary administration forms, as a mixture with conventional
35 pharmaceutical supports, to animals and to human beings. The appropriate unitary administration forms comprise the forms by the oral route such as compressed tablets, gelatin capsules, powders, granules and oral solutions or suspensions, sublingual

and buccal administration forms, subcutaneous, intramuscular, intravenous, intranasal or intraocular administration forms, and rectal administration forms.

When a solid composition in the form of compressed tablets is prepared, the principal active ingredient is mixed with a pharmaceutical vehicle such as gelatin, starch, lactose, magnesium stearate, talc, gum arabic or the like. It is possible to coat the compressed tablets with sucrose or other appropriate materials or alternatively it is possible to treat them in such a way that they have a prolonged or delayed activity and that they liberate a predetermined quantity of active principle in a continuous manner.

A preparation in gelatin capsules is obtained by mixing the active ingredient with a diluent and by pouring the mixture obtained into soft or hard gelatin capsules.

A preparation in syrup or elixir form can contain the active ingredient together with a preferably acałoric sweetener, methylparaben and propylparaben as antiseptic, as well as a taste-imparting agent and an appropriate colourant.

The water-dispersible powders or granules can contain the active ingredient as a mixture with dispersing agents or wetting agents, or suspending agents, such as polyvinylpyrrolidone, as well as with sweeteners or taste corrigents.

For rectal administration, recourse is had to suppositories which are prepared with binders melting at rectal temperature, for example cocoa butter or polyethylene glycols.

For parenteral, intranasal or intraocular administration, aqueous suspensions, isotonic saline solutions or sterile and injectable solutions which contain pharmacologically compatible dispersing agents and/or wetting agents, for example propylene glycol or butylene glycol, are used.

For transmucosal administration, the active principle can be formulated in the presence of a promoter such as a bile salt, a hydrophilic polymer such as, for example, hydroxypropylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, ethylcellulose, carboxymethylcellulose, dextran, polyvinylpyrrolidone, pectins, starches, gelatin,

casein, acrylic acids, acrylic esters and their copolymers, vinyl polymers or copolymers, vinyl alcohols, alkoxy polymers, polyethylene oxide polymers, polyethers or a mixture thereof.

5 The active principle can also be formulated in the form of microcapsules, optionally with one or more supports or additives.

The active principle can also be present in complex form with a cyclodextrin, for example, α -, β - or γ -cyclodextrin, 2-hydroxypropyl- β -cyclodextrin or methyl- β -cyclodextrin.

10 The active principle can also be liberated by a balloon containing it or by an endovascular extensor introduced into the blood vessels. The pharmacological efficacy of the active principle is thus unaffected.

15 Administration by the subcutaneous route is the preferred route.

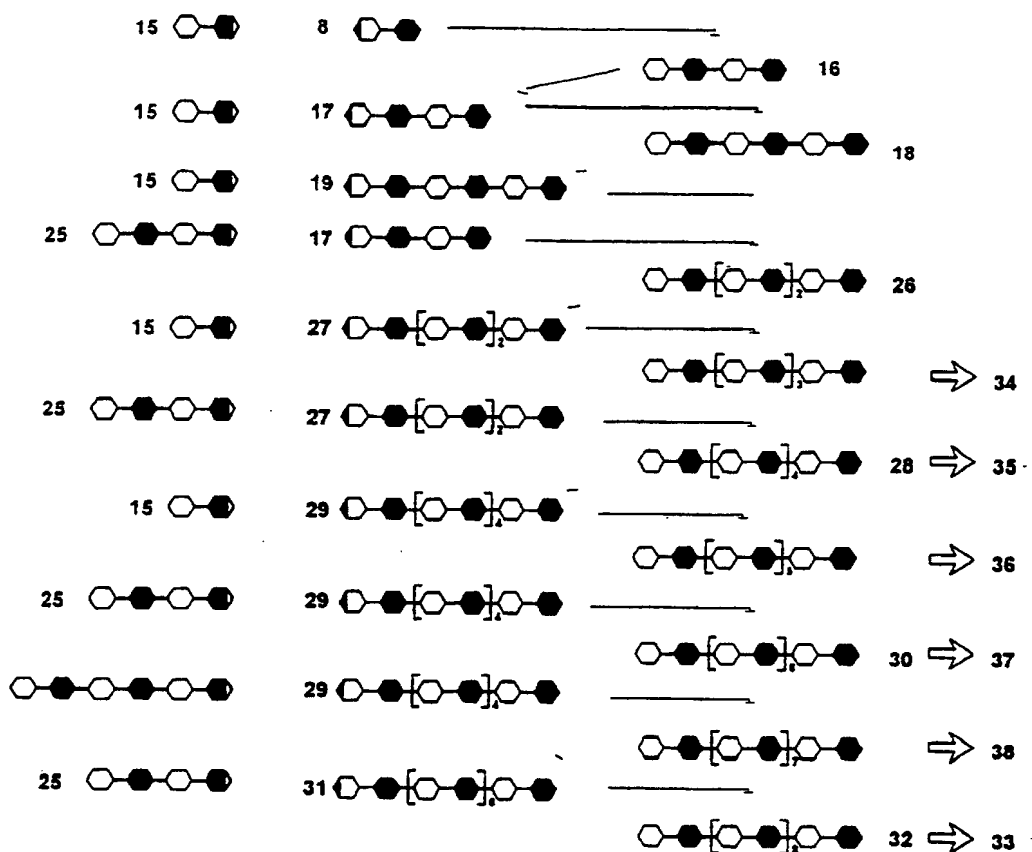
The following METHODS, PREPARATIONS and SCHEMES illustrate the synthesis of the different intermediates useful in obtaining polysaccharides according to the invention.

20 The EXAMPLES below likewise illustrate the invention without, however, limiting it.

For a better comprehension of the process according to the invention, the obtainment of the compounds (I) can be schematized as follows:

25

SCHEME 1



Strategy employed for the synthesis of oligomers of the
 disaccharide methyl 4-O-(2,3-di-O-methyl- α -L-idopyranosyl-
 uronate)-2,3,6-tri-O-sulpho- α -D-glucopyranoside. By condensing
 the imidates (left column, white triangle at the reducing end)
 and the glycosyl acceptors (centre column, black triangle at the
 non-reducing end) completely protected oligosaccharides of the
 right column are obtained. The latter are then deprotected and
 functionalized to yield the compounds of EXAMPLE 1 and of TABLE
 I.

All the compounds described below are homogeneous on
 thin-layer chromatography (TLC) and have spectral properties in
 agreement with their structure. The melting points are
 determined in capillary tubes with the aid of a Mettler
 apparatus, and are uncorrected. The rotatory powers are

measured with the aid of a Perkin-Elmer 241 polarimeter at $22 \pm 3^\circ\text{C}$. The purity of the compounds is checked by TLC on Silica Gel 60® F₂₅₄ (E. Merck) with detection by carbonization in the presence of sulphuric acid. Unless mentioned otherwise, column chromatography was carried out on Silica Gel 60® 40-63 or 63-200 μm (E. Merck). The ^1H NMR spectra are recorded on Bruker AC 200, AM 250, AC 300 or AM 500 apparatuses, on solutions of the products in CDCl_3 or D_2O . Before analysis in D_2O , the samples are passed through a column of Chelex® ion exchange resin (Bio-Rad) and then lyophilized three times in D_2O . The chemical shifts are relative to the external TMS when the spectra are recorded in CDCl_3 , and to external TSP when the spectra are recorded in D_2O . The mass spectrometric analyses are carried out in a ZAB-2E instrument (Fisons). The elemental analyses are carried out in a Fisons analyser.

The following abbreviations are used:

TBDMS: *tert*-butyldimethylsilyl; Lev: levulinyll; Bn: benzyl; Bz: benzoyl; MCA: chloroacetyl; CCM: thin-layer chromatography; Olm: trichloroacetimidyl; LSIMS: are the initials of *Liquid Secondary Ion Mass Spectrometry*; ESIMS: are the initials of *Electron Spray Ionization Mass Spectrometry*; TMS: trimethylsilyl; TSP: sodium trimethylsilyltetraduteriopropionate; Tf: triflate; MS: molecular sieve.

Dowex®, Sephadex®, Chelex®, Gel 60® are trade marks.

In the METHODS, the PREPARATIONS and in the EXAMPLES described below, general working methods concerning the cleavage of the levulinic esters, the catalytic coupling of the imidates, the deprotection and the sulphation of the oligo- and of the polysaccharides by hydrogenolysis of the esters or of the benzyl ethers, the saponification of the esters or alternatively the sulphations can be carried out by applying the general methods below to the appropriate intermediates.

GENERAL METHODS**METHOD 1.** *Cleavage of the Lev group.*

A solution of hydrazine hydrate (1 M in 3:2 pyridine/acetic acid) is added (5 ml/mmol) to a cooled solution (0°C) in pyridine (5 ml/mmol) of the compound to be treated. After 15-30 minutes (TLC), the solution is concentrated. The residue is dissolved in ethyl acetate, washed with water, a 10% solution of potassium hydrogensulphate, a 2% solution of sodium hydrogencarbonate and with water, and is dried (sodium sulphate) and concentrated.

METHOD 2. *Coupling to the imidates catalysed by tert-butyldimethylsilyl triflate.*

tert-Butyldimethylsilyl triflate (0.5 mol/mol of imidate) is added dropwise, under argon, to a stirred and cooled solution (-20°C) of the acceptor alcohol and of the donor imidate in toluene (35 ml/mmol), in the presence of 4 Å molecular sieve in powder form. After 15-30 minutes (TLC), solid sodium hydrogencarbonate is introduced with stirring. After 5 minutes, toluene is added, the solution is filtered and washed with a solution of 2% sodium hydrogencarbonate and with water, and is dried (sodium sulphate) and concentrated.

METHOD 3. *Coupling to the imidates catalysed by trimethylsilyl triflate.*

Trimethylsilyl triflate (0.04 M in toluene; 0.06 mol/mol of imidate) is added dropwise, under argon, to a stirred and cooled solution (-20°C) of the acceptor alcohol and of the donor imidate, in toluene (15 ml/mmol), in the presence of 4 Å molecular sieve in powder form. After 15-30 minutes (TLC), solid sodium hydrogencarbonate is introduced with stirring. After 5 minutes, toluene is added, the solution is filtered and washed with a solution of 2% sodium hydrogencarbonate and with water, and is dried (magnesium sulphate) and concentrated.

METHOD 4. *Deprotection and sulphation of the oligo- and polysaccharides.*

Hydrogenolysis of the benzyl ethers and benzyl esters. A solution of compound (5 mg/ml) in dimethylformamide or methanol is stirred for 2-6 hours (TLC checking) under a

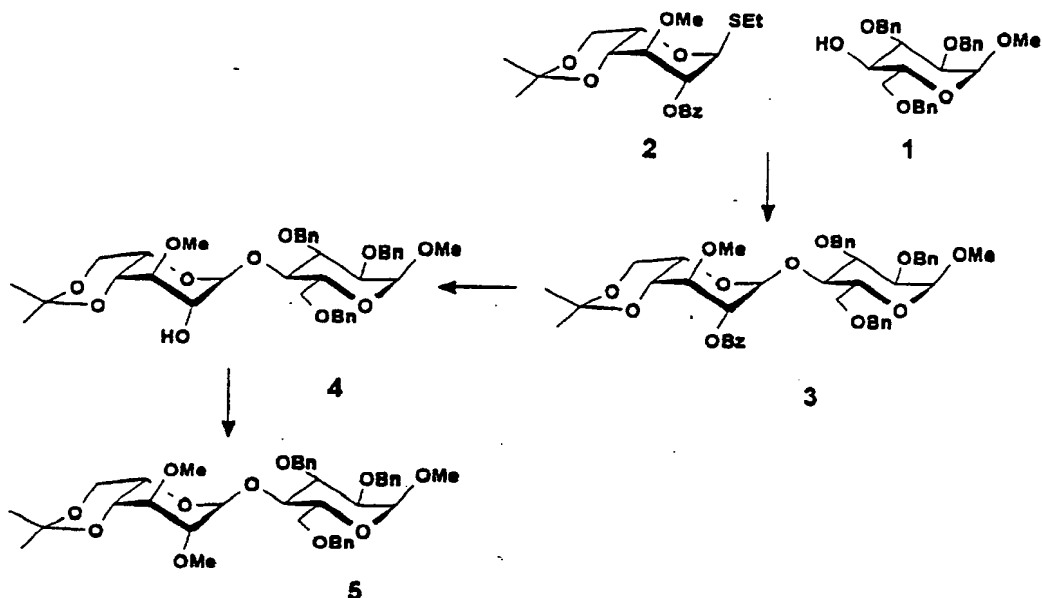
hydrogen atmosphere (5 bar) in the presence of 10% Pd/C catalyst (2 x mass of the compound). After filtration, the product is employed directly in the following step.

Saponification of the esters. An aqueous solution of 5 M sodium hydroxide is added (in a quantity such that the concentration of sodium hydroxide is 0.5 M at the end of addition) to a solution of an ester in methanol (150 ml/mmol). After 2-5 hours (TLC), water is introduced followed by the resin Dowex® 50 H⁺ to pH 1-2. After filtration and concentration, the residue is passed through a column of Sephadex® G-25 gel (1.6 x 115 cm) eluted with water. The completely deprotected compound is then obtained after lyophilization. At this stage, it is checked by ¹H NMR that all the protective groups have been removed. If it is necessary, the product is again subjected to hydrogenation and/or saponification.

Sulphation. Pyridine/sulphur trioxide complex (5 mmol/mmol of hydroxyl function) is added to a solution in dimethylformamide (10 mg/ml) of the compound to be sulphated. After one day at 55° C, the solution is placed on the top of a column of Sephadex G-25 (1.6 x 115 cm) eluted with 0.2 M sodium chloride. The fractions containing the product are concentrated and desalted by using the same column eluted with water. The final compound is obtained after lyophilization.

SCHEME 2

Synthesis of the disaccharide 5



PREPARATION 1

5 **Methyl 4-O-(2-O-benzoyl-4,6-isopropylidene-3-O-methyl-α-L-idopyranosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (3).**

A solution of triflic acid in toluene (0.15 M, 0.27 ml) is added, with stirring under argon, to a cooled solution (-20°C) of ethyl 2-O-benzoyl-4,6-O-isopropylidene-3-O-methyl-1-thio-α-L-idopyranoside 2 (Jaurand, G. et al., BioMed. Chem. Lett. 1992, 2, 897-900) (1.1 g, 2.87 mmol), of 1 (Garegg P.J., Hultberg H., Carbohydr. Res. 93, 1981 C10-C11) (1.34 g, 2.87 mmol) and of N-iodosuccinimide (1.61 g, 7.2 mmol) in toluene (40 ml) containing 4 Å molecular sieves in powder form. 10 The same quantity of acid is added after 25 and 50 minutes. After 1.5 h, solid sodium bicarbonate (20 mg) is introduced, and, 15 minutes later, the solution is filtered, diluted with dichloromethane, washed with a sodium thiosulphate solution and water, dried (sodium sulphate) and evaporated. The crude 15 product thus obtained (2.49 g) is used directly for the preparation of 4.

After column chromatography (3:1 cyclohexane/ethyl acetate),

compound **3** is obtained pure. TLC, $R_f = 0.36$, 3:1 cyclohexane/ethyl acetate; $[\alpha]_D + 31$ ($c = 1$, dichloromethane) ESI MS, positive mode: $m/z + NaCl$, 345 ($M+Na$)⁺; + KF, 361 ($M+K$)⁺. ¹H NMR (CDCl₃) δ 7.17-7.35 (m, 20H, 4Ph), 5.10 (d, 1H, H-1'), 4.60 (d, 1H, $J = 3.0$ Hz, H-1), 3.37 (s, 3H, OMe), 1.95; 2.04; 2.09 (3s, 9H, 3Ac), 1.24; 1.33 (2s, 6H, :C(CH₃)₂).

Anal. Calculated for C₄₅H₅₂O₁₂ (784.86): C, 68.86, H, 6.68. Found: C, 68.61; H, 6.77.

PREPARATION 2

Methyl 2,3,6-tri-O-benzyl-4-O-(4,6-isopropylidene-3-O-methyl- α -L-idopyranosyl)- α -D-glucopyranoside (4).

A 2 M solution of sodium methoxide (2.2 ml, 4.4 mmol) is added to a solution of compound **3** (2.34 g) in a 1:1 methanol/dichloromethane mixture (13 ml). After 2.5 hours at ambient temperature, the mixture is neutralized with Dowex® 50 resin (H⁺), filtered and concentrated, to give **4** (1.74 g; 86% with respect to **1** and **2**) after column chromatography (3:1 then 2:1 cyclohexane/ ethyl acetate); $[\alpha]_D + 23$ ($c = 1$, dichloromethane). ESI MS, positive mode: $m/z + NaCl$, 703 ($M+Na$)⁺; + KF, 719 ($M+K$)⁺. ¹H NMR (CDCl₃) δ 7.31-7.21 (m, 15H, 3Ph), 4.94 (d, 1H, H-1'), 4.60 (d, 1H, $J = 3.6$ Hz, H-1), 3.44; 3.36 (2s, 6H, OMe); 3.06 (dd, 1H, $J = 3.6$ Hz, $J = 12.2$ Hz, H-6'), 1.31; 1.28 (2s, 6H, :C(CH₃)₂).

Anal. Calculated for C₃₈H₄₈O₁₁ (680.76): C, 67.04; H, 7.11. Found: C, 67.05; H, 7.16.

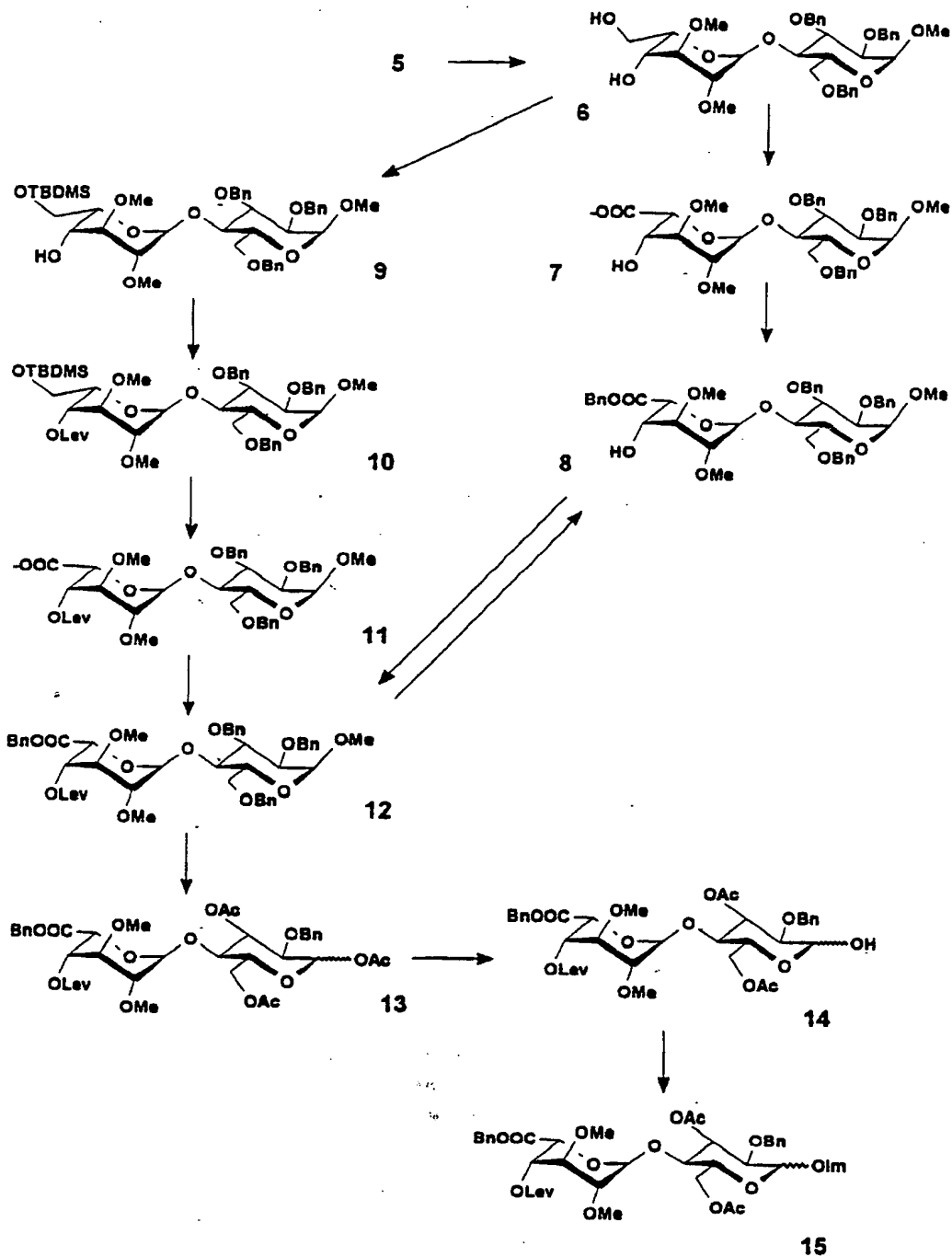
PREPARATION 3

Methyl 2,3,6-tri-O-benzyl-4-O-(4,6-isopropylidene-2,3-di-O-methyl- α -L-idopyranosyl)- α -D-glucopyranoside (5).

Methyl iodide (3.2 ml, 50.8 mmol) is added, at 0°C, to a solution of **4** (26.6 g, 39.1 mmol) and of sodium hydride (1.48 g, 58.7 mmol) in dimethylformamide (60 ml). A new addition of methyl iodide (1.6 ml, 25.4 mmol) and of sodium hydride (0.74 g, 29.3 mmol) is carried out after 5 hours at ambient temperature. After 1 night, methanol (10 ml) is introduced dropwise, and after 1.5 hours the reaction mixture

is concentrated. The product is extracted with ethyl acetate (1.5 l). The solution is washed with water, dried (sodium sulphate) and concentrated. The crude compound **5** thus obtained (32.1 g) is used as such in the following step. TLC, $R_f = 0.55$,
5 3:2 cyclohexane-ethyl acetate.

SCHEME 3



Synthesis of the base disaccharides for the preparation of oligomers of methyl 4-O-(2,3-di-O-methyl- α -L-idopyranosyluronate)-2,3,6-tri-O-sulpho- α -D-glucopyranoside.

PREPARATION 4

Methyl 2,3,6-tri-O-benzyl-4-O-(2,3-di-O-methyl- α -L-idopyranosyl)- α -D-glucopyranoside (6).

An aqueous solution of trifluoroacetic acid (70%, 5 43 ml) is added dropwise during the course of 10 minutes to a solution of the above crude compound (32.1 g) in dichloromethane (215 ml). After 25 minutes at ambient temperature, the solution is diluted with dichloromethane (1 l), washed with a saturated aqueous solution of sodium 10 hydrogencarbonate and with water, and dried (sodium sulphate). The crude compound 6 obtained after concentration (27.5 g) is used as such in the following step. TLC, R_f = 0.29, 2:3 cyclohexane-ethyl acetate.

PREPARATION 5

15 **Methyl 2,3,6-tri-O-benzyl-4-O-(6-O-tert-butyl-dimethylsilyl-4-O-levulinyl-2,3-di-O-methyl- α -L-idopyranosyl)- α -D-glucopyranoside (10).**

A solution of 6 (1.7 g), of triethylamine (0.54 ml, 3.8 mmol), of 4-dimethylaminopyridine (38 mg, 0.3 mmol) and of 20 tert-butyldimethylsilyl chloride (0.54 g, 3.6 mmol), in methylene chloride (6 ml) is heated at 50°C for 3 hours to give 9 which is not isolated. After cooling to ambient temperature, levulinic anhydride (0.771 g, 3.6 mmol), triethylamine (0.50 ml, 3.6 mmol) and 4-dimethylaminopyridine (59 mg, 25 0.48 mmol) are added. After 4 hours, the mixture is diluted with methylene chloride, and washed successively with an aqueous solution of potassium hydrogensulphate, water, a saturated aqueous solution of sodium hydrogencarbonate and water, dried (sodium sulphate) and concentrated to give 10 30 (2.45 g) which is used as such in the following step. TLC, R_f 0.5, 12:1 cyclohexane/ethyl acetate.

PREPARATION 6

Methyl 2,3,6-tri-O-benzyl-4-O-(benzyl 4-O-levulinyl-2,3-di-O-methyl- α -L-idopyranosyluronate)- α -D-glucopyranoside 35 (12).

A solution of chromium trioxide (0.64 g, 6.4 mmol) in aqueous sulphuric acid (3.5 M, 2.7 ml) is added slowly to a

cooled solution (0°C) of **10** (2.45 g) in acetone (18 ml). After 5 hours, methylene chloride is introduced, then the mixture is poured into ice water, stirred vigorously, and washed with water to neutral pH and dried (sodium sulphate). Concentration gives **11** (2.45 g) in syrup form. TLC, R_f 0.56, 12:1 methylene chloride/methanol. This product is then dissolved in dimethylformamide (19 ml) and treated for one night at ambient temperature with benzyl bromide (2.9 ml, 12 mmol). Methanol (1.5 ml) is added and the product is extracted with ether, washed with water, dried and concentrated. After column chromatography (2:1 then 3:2 cyclohexane/ethyl acetate) **12** is obtained (1.07 g). TLC, R_f 0.53, 5:1 methylene chloride/ethyl acetate.

PREPARATION 7

Methyl 2,3,6-tri-O-benzyl-4-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)- α -D-glucopyranoside (8).

The disaccharide **12** (0.65 g, 0.76 mmol) treated according to general method 1 gives **8** (0.52 g, 91%) after column chromatography (2:1 then 3:1 cyclohexane/ethyl acetate). $[\alpha]_D + 34$ ($c = 0.97$, methylene chloride).

PREPARATION 8

1,3,6-tri-O-Acetyl-2-O-benzyl-4-O-(benzyl 4-O-levulinyl-2,3-di-O-methyl- α -L-idopyranosyluronate)-D-glucopyranose (13).

Trifluoroacetic acid (28 ml, 0.364 mol) is added to a solution of **12** (7.8 g, 9.1 mmol) in acetic anhydride (194 ml, 2.06 mol) and acetic acid (7.8 ml, 0.136 mol). After heating to 60°C for 4 hours, the solution is cooled to 0°C and water (30 ml) is introduced dropwise, followed by triethylamine (69 ml). After evaporation, the residue is dissolved in dichloromethane, washed with a saturated solution of sodium hydrogencarbonate and water, dried (sodium sulphate) and concentrated. Column chromatography (5:1 dichloromethane/ethyl acetate) gives a mixture ($\alpha/\beta=8/2$) of the anomers of **13** (4.7 g, 67%). TLC, R_f 0.35; 3:2 cyclohexane-acetone. ^1H NMR (CDCl_3) δ 7.37-7.20 (m, 10H, 2Ph), 6.30 (d, $J = 3.6$ Hz, H-1 α), 5.62 (d, $J = 7.6$ Hz, H-1 β), 5.04 (t, 1H, H-4'), 3.45; 3.41 (2s, 6H, 2 OMe),

2.6-2.3 (m, 4H, $O(C:O)CH_2CH_2(C:O)CH_3$), 2.15; 2.12; 2.06; 1.94; 1.88 (5s, 12H, 3 Ac and $O(C:O)CH_2CH_2(C:O)CH_3$).

PREPARATION 9

**3,6-di-O-Acetyl-2-O-benzyl-4-O-(benzyl 4-O-levulinyl-
5 2,3-di-O-methyl- α -L-idopyranosyluronate)-D-glucopyranose (14).**

A solution of ethanolamine (1.3 ml, 21.6 mmol) and of **13** (4.25 g, 5.28 mmol) in tetrahydrofuran (80 ml) is left for one night at 4°C. Ethanolamine (0.65 ml, 10.8 mmol) is then added, then the mixture is left at ambient temperature for 3 h.
10 After cooling to 0°C, 1 M hydrochloric acid is added to acid pH, then dichloromethane (150 ml). The solution is washed with water, dried and concentrated. Column chromatography (2:1 then 1:1 dichloromethane/ethyl acetate) gives **14** (3 g, 74%). TLC, R_f 0.21, 1:1 toluene/ethyl acetate. $[\alpha]_D^{25} + 3$ (c = 1, 15 dichloromethane). LSIMS, positive mode: m/z thioglycerol + NaCl, 769 (M+Na)⁺; thioglycerol + KF, 785 (M+K)⁺. ¹H NMR (CDCl₃) δ 7.36-7.25 (m, 10H, 2Ph), 5.21 (d, J = 3.5 Hz, H-1 α), 4.98 (d, 1H, J = 3.4 Hz, H-1'), 4.79 (d, J = 8 Hz, H-1 β), 3.45; 3.42 (2s, 6H, 2 OMe), 2.56-2.23 (m, 4H, $O(C:O)CH_2CH_2(C:O)CH_3$), 2.23; 20 2.12; 1.94; 1.88 (4s, 9H, 2 Ac, α and β $O(C:O)CH_2CH_2(C:O)CH_3$).

Anal. Calculated for C₃₇H₄₆O₁₆ (746.72); C, 59.51; H, 6.21. Found: C, 58.87, H, 6.13.

PREPARATION 10

**3,6-di-O-Acetyl-2-O-benzyl-4-O-(benzyl 4-O-levulinyl-
25 2,3-di-O-methyl- α -L-idopyranosyluronate)-D-glucopyranose
trichloroacetimidate (15).**

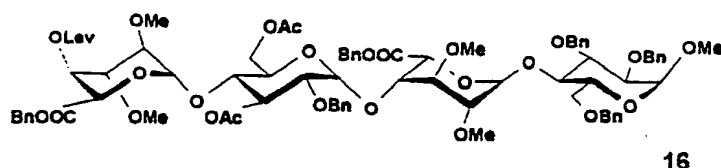
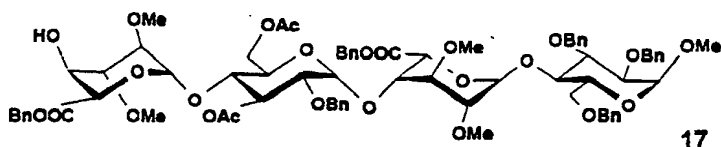
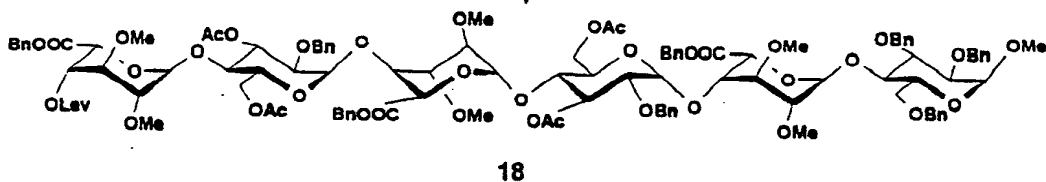
A mixture of trichloroacetonitrile (0.7 ml, 6.92 mmol), of **14** (1.03 g; 1.38 mmol) and of potassium carbonate (191 mg; 2.21 mmol) in methylene chloride (26 ml) is stirred for
30 1.5 hours at ambient temperature. The solution is then filtered and concentrated. Column chromatography (4:1 toluene/acetone) gives **15** (1.16 g; 94%). TLC, R_f 0.31 and 0.48, 2:3 cyclohexane/ethyl acetate. LSIMS, positive mode: m/z thioglycerol + LiCl, 896 (M+Li)⁺; thioglycerol + NaCl, 912
35 (M+Na)⁺; thioglycerol + KF, 928 (M+K)⁺. ¹H NMR (CDCl₃) δ 8.67 (s, NH- β), 8.60 (s, NH- α), 7.37-7.22 (m, 10H, 2Ph); 6.44 (d, J =

3.6 Hz, H-1 α), 5.83 (d, J = 7.3 Hz, H-1 β), 3.47; 3.44; 3.42; 3.40 (4s, 6H, 2 OMe), 2.7-2.2 (m, 4H, O(C:O)CH₂CH₂(C:O)CH₃), 2.15; 2.08; 1.94; 1.88 (4s, 9H, α and β Ac, and O(C:O)CH₂CH₂(C:O)CH₃).

5

SCHEME 4

8 + 15

CH₂Cl₂; MS; TMSOTfNH₂NH₂; pyridine-AcOH+ 15 CH₂Cl₂; MS; TMSOTf

10 Synthesis of the hexasaccharide **18**. The preparation of
oligomers of larger size is carried out according to the same
strategy (cleavage of the Lev group to obtain a glycosyl
acceptor, coupling with a di-, tetra- or hexasaccharide imidate
- as indicated in Scheme 1 - and finally deprotection and
sulphation). The Lev group of **18** is selectively eliminated to
15 give the acceptor hexasaccharide **19** (SCHEME 1).

PREPARATION 11

Methyl (1-4)-O-(benzyl 4-O-levulinyl-2,3-di-O-methyl- α -
L-idopyranosyluronate)-(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-
glucopyranosyl)-(1-4)-O-(benzyl 2,3-di-O-methyl- α -L-
20 idopyranosyluronate)-2,3,6-tri-O-benzyl- α -D-glucopyranoside
(**16**).

A mixture of **8** (2.90 g, 3.83 mmol) and **15** (4.16 g, 4.67 mmol) is treated according to method 2. Column chromatography (1:1 cyclohexane/ethyl acetate) gives pure **16** (3.2 g; 54%). TLC, R_f 0.52, 2:3 cyclohexane/ethyl acetate. ^1H NMR (CDCl_3) δ 7.21-7.36 (m, 30H, 6Ph), 5.27 (d, 1H, H-1, non-reducing unit), 5.14 (d, 1H, H-1 "central next to non-reducing" unit), 4.90 (d, 1H, H-1, "central next to reducing" unit), 4.56 (d, 1H, H-1 reducing unit), 3.43; 3.39; 3.35; 3.25 (5s, 15H, 5 OMe), 2.25-2.60 (m, 4H, $\text{O}(\text{C}=\text{O})\text{CH}_2\text{CH}_2(\text{C}=\text{O})\text{CH}_3$), 2.12; 2.00; 1.91 (3s, 9H, 2Ac and $\text{O}(\text{C}=\text{O})\text{CH}_2\text{CH}_2(\text{C}=\text{O})\text{CH}_3$).

PREPARATION 12

Methyl (1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)-(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-glucopyranosyl)-(1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)-2,3,6-tri-O-benzyl- α -D-glucopyranoside (**17**).

Compound **16** (1 g; 0.672 mmol) is treated according to method 1 to give **17** quantitatively after column chromatography (1:1 cyclohexane/acetone).

PREPARATION 13

Methyl (1-4)-O-(benzyl 4-O-levulinyl-2,3-di-O-methyl- α -L-idopyranosyluronate)-[(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-glucopyranosyl)-(1-4)-O-(L-benzyl 2,3-di-O-methyl- α -idopyranosyluronate)]₂-2,3,6-tri-O-benzyl- α -D-glucopyranoside (**18**).

A mixture of **15** (386 mg, 434 μmol) and **17** (500 mg; 360 μmol) is treated according to method 2. Column chromatography (Sephadex® LH 20, 195 x 3.7 cm; 1:1 dichloromethane/ethanol) gives the hexasaccharide **18** (495 mg; 64%). TLC, R_f 0.36; 10:1 dichloromethane/ acetone.

^1H NMR (CDCl_3) δ : 5.23; 5.12; 5.10; 4.92; 4.89; 4.56 ppm.

PREPARATION 14

Methyl (1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)-[(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-

glucopyranosyl)-(1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)],-2,3,6-tri-O-benzyl- α -D-glucopyranoside (19).

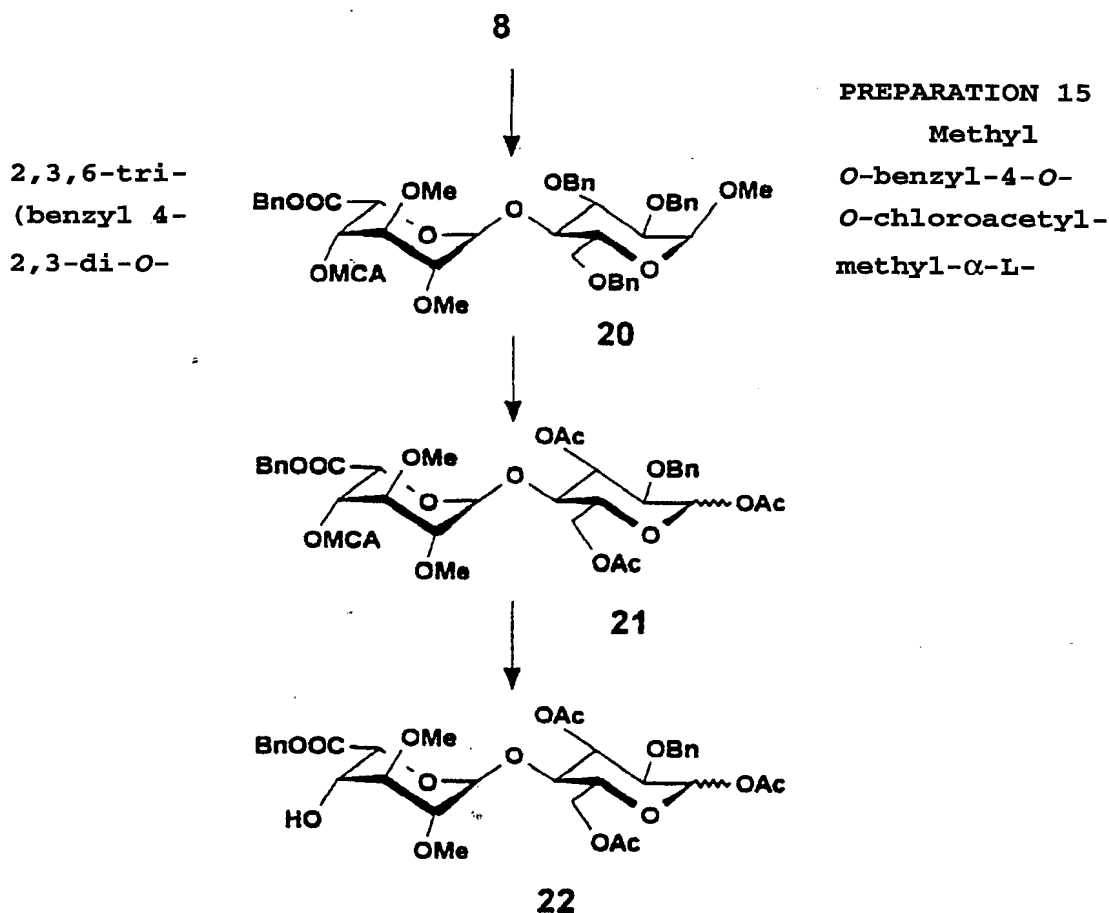
Compound 18 (485 mg; 229 μ mol) is treated according to method 1. Column chromatography (10:1 dichloromethane/acetone) gives 19 (392 mg; 85%). TLC, R_f 0.38, 10:1 dichloromethane/acetone.

SCHEME 5

Synthesis of the acceptor disaccharide 22

10

15



idopyranosyluronate)- α -D-glucopyranoside (20).

A mixture of 8 (326 mg, 0.43 mmol), of chloroacetic anhydride (103 g, 0.6 mmol), of 4-dimethylaminopyridine (5.3 mg, 42 μ mol) and of triethylamine (90 μ l, 64 μ mol) in 45 dichloromethane (96 ml) is stirred at ambient temperature for

30 minutes. Methanol (0.5 ml) is then added, and the solution is diluted with dichloromethane, washed with water, dried (sodium sulphate) and concentrated. Column chromatography (2:3 then 1:2 cyclohexane/ ether) gives pure **20** (242 mg, 67%). TLC, R_f 0.35, 1:2 cyclohexane/ether. ^1H NMR (CDCl_3) δ 7.36-7.19 (m, 20H, 4Ph), 5.20 (d, 1H, H-1'), 4.56 (d, 1H, H-1), 3.70 and 3.36 (AB system, $J = 15.3$ Hz, $\text{ClCH}_2(\text{C}=\text{O})\text{O}$), 3.49, 3.35; 3.26 (3s, 3 OCH_3).

10 **PREPARATION 16**

1,3,6-tri-O-Acetyl-2-O-benzyl-4-O-(benzyl 4-O-chloro-acetyl-2,3-di-O-methyl- α -L-idopyranosyluronate)- α,β -D-glucopyranose (21).

A solution of trifluoroacetic acid (183 μl , 2.4 mmol) is added to a solution of **20** (50 mg, 0.06 mmol) in acetic anhydride (1.28 ml, 13.5 mmol) and acetic acid (52 μl , 0.9 mol). After heating at 60°C for 4 hours, the solution is cooled to 0°C and neutralized with triethylamine. After evaporation, column chromatography of the residue (1:2 then 2:5 cyclohexane/ether) gives a mixture ($\alpha/\beta = 8/2$) of the anomers of **21** (28 mg, 60%). TLC, R_f 0.31, 2:5 cyclohexane/ether. ^1H NMR (CDCl_3) δ 7.20-7.35 (m, 10H, 2Ph), 6.30 (d, $J = 3.6$ Hz, H-1 α), 5.63 (d, $J = 8.1$ Hz, H-1 β), 3.39 and 3.46 (2s, 2 OCH_3).

25 **PREPARATION 17**

1,3,6-tri-O-Acetyl-2-O-benzyl-4-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)- α,β -D-glucopyranose (22).

Thiourea (678 mg; 8.9 mmol) is added to a solution of **21** (1.71 g; 2.23 mmol) in a mixture of pyridine (108 ml) and ethanol (22 ml), and the mixture is heated at 110°C for 30 minutes. After cooling and evaporation, the residue is dissolved in dichloromethane. The solution is washed with the aid of a saturated aqueous solution of sodium hydrogencarbonate, then a 5% potassium hydrogensulphate solution, dried (sodium sulphate) and concentrated. Column chromatography (1:1, then 1:2 cyclohexane/ethyl acetate) gives pure **22** (1.17 g; 76%). TLC, R_f 0.34; 3:1 dichloromethane/ether.

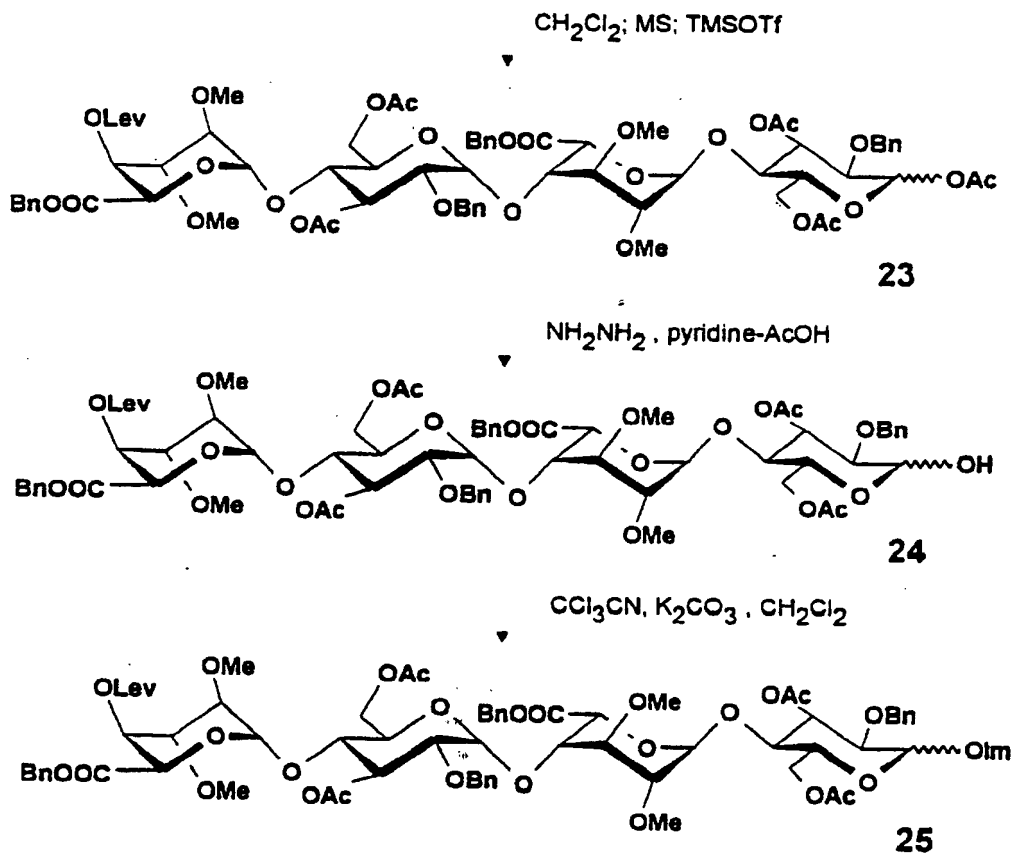
^1H NMR (CDCl_3) δ 7.36-7.20 (m, 10H, 2Ph), 6.30 (d, $J = 3.6$ Hz, H-1 α), 5.65 (d, $J = 8$ Hz, H-1 β), 4.90 (1H, H-1'), 3.41 and 3.40 (2s, 2 OCH_3)

5

SCHEME 6

Synthesis of the tetrasaccharide imidate 25

15 + 22



10

PREPARATION 18

(1-4)-O-(benzyl 4-O-Levulinyl-2,3-di-O-methyl- α -L-idopyranosyluronate)-(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-glucopyranosyl)-(1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)-1,3,6-tri-O-acetyl-2-O-benzyl- α,β -D-

15

glucopyranose (23).

A mixture of **15** (1.5 g, 1.7 mmol) and **22** (1.18 g; 1.7 mmol) is treated according to method 3 (by replacing the toluene with dichloromethane). Gel permeation chromatography on a column of LH-20, equilibrated in dichloromethane/ethanol 1:1, gives pure **23** (1.75 g; 73%). $[\alpha]_D + 19$ (c = 0.9, dichloromethane). LSIMS, positive mode: m/z thioglycerol + NaCl, 1441 (M+Na)⁺; thioglycerol + KF, 1457 (M+K)⁺.

¹H NMR (CDCl₃) δ : 6.27; 5.45; 5.10; 4.96; 4.90 ppm.

Anal. Calc. for C₇₁H₈₆O₃₀ (1419.46); C, 60.08; H, 6.11. Found: C, 60.06; H, 6.40.

PREPARATION 19

(1-4)-O-(benzyl 4-O-Levulinyl-2,3-di-O-methyl- α -L-idopyranosyluronate)-(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-glucopyranosyl)-(1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)-3,6-di-O-acetyl-2-O-benzyl- α,β -D-glucopyranose (**24**).

Ethanolamine (80 μ l; 1.31 mmol) is added to a solution of the tetrasaccharide **23** (465 mg; 327 μ mol) in tetrahydrofuran (5 ml), then the solution is left overnight at 4°C. After neutralization with hydrochloric acid (1 M; 2 ml), dichloromethane (20 ml) is added, then the solution is washed with water, dried (sodium sulphate) and concentrated. Column chromatography (3:1 toluene/acetone) gives **24** (326 mg; 79%). TLC, R_f 0.33, 3:1 toluene/acetone. LSIMS, positive mode: m/z thioglycerol + NaCl, 1399 (M+Na)⁺; thioglycerol + KF, 1415 (M+K)⁺.

¹H NMR (CDCl₃) δ : 5.18; 5.10; 4.96; 4.93; 4.75

PREPARATION 20

(1-4)-O-(benzyl 4-O-Levulinyl-2,3-di-O-methyl- α -L-idopyranosyluronate)-(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-glucopyranosyl)-(1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)-3,6-di-O-acetyl-2-O-benzyl- α,β -D-glucopyranose trichloroacetimidate (**25**).

A mixture of trichloroacetonitrile (151 μ l; 1.5 mmol), of the tetrasaccharide **24** (343 mg; 249 μ M) and of potassium

carbonate (62 mg; 448 μ mol) in dichloromethane (2 ml) is stirred for one night at ambient temperature. Dichloromethane is added, and after filtration the solution is concentrated. Column chromatography (3:1 toluene/acetone) gives **25** (346 mg; 91%).

TLC, R_f 0.42; 0.63, 2:1 dichloromethane/ethyl acetate.

^1H NMR (CDCl_3) δ : 8.67 (s, NH-b), 8.59 (s, NH-a), 7.40-7.20 (m, 10H, 2Ph), 6.40 (d, J = 3.5 Hz, H-1 α), 5.90 (d, J = 7.5 Hz, H-1 β), 3.45; 3.44; 3.42; 3.40; 3.39; 3.37 (6s, 12H, 4 OMe), 2.7-2.2 (m, 4H, $\text{O}(\text{C}=\text{O})\text{CH}_2\text{CH}_2(\text{C}=\text{O})\text{CH}_3$), 2.12; 2.08; 2.07; 2.04; 2.02; 1.91; 1.89 (7s, 15H, 4 Ac, and $\text{O}(\text{C}=\text{O})\text{CH}_2\text{CH}_2(\text{C}=\text{O})\text{CH}_3$). NH-a and NH-b designate the signals obtained for each of the syn and anti isomers.

PREPARATION 21

Methyl (1-4)-O-(benzyl 4-O-Levulinyl-2,3-di-O-methyl- α -L-idopyranosyluronate)-[(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-glucopyranosyl)-(1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)]₃-2,3,6-tri-O-benzyl- α -D-glucopyranoside (26).

Starting from **17** and **25**. - A mixture of **17** and **25** (459 mg, 0.3 mmol) is treated according to Method 3. Column chromatography (Sephadex® LH 20, 195 x 3.7 cm; 1:1 dichloromethane/ethanol), followed by silica gel column chromatography (3:2 cyclohexane-acetone) gives pure **26** (420 mg; 59%). $[\alpha]_D^{20}$ (c = 0.20, dichloromethane). LSIMS, positive mode: m/z thioglycerol + NaCl, 2771 ($M+\text{Na}$)⁺; thioglycerol + KF, 2787 ($M+\text{K}$)⁺.

^1H NMR (CDCl_3) δ : 5.26; 5.14; 5.10; 4.93; 4.92; 4.90; 4.56

PREPARATION 22

26 starting from **15** and **19**. - A mixture of **15** (332 mg; 373 μ mol) and **19** (377 mg; 186 μ mol) is treated according to method 2. Column chromatography (Sephadex® LH 20, 195 x 3.7 cm; 1:1 dichloromethane/ethanol) gives the pure octasaccharide **26**

(460 mg; 90%).

PREPARATION 23

Methyl (1-4)-O-(benzyl 2,3-di-O-Methyl- α -L-idopyranosyluronate)-[(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl- α -L-benzyl idopyranosyluronate)],-2,3,6-tri-O-benzyl- α -D-glucopyranoside (27).

Compound 26 (275 mg; 100 μ mol) is treated according to method 1 to give 27 (265 mg; 96%); $[\alpha]_D + 27$ ($c = 0.56$, dichloromethane). LSIMS, positive mode: m/z thioglycerol + NaCl, 2673 ($M+Na$)⁺; thioglycerol + KF, 2689 ($M+K$)⁺.

¹H NMR (CDCl₃) δ : 5.26; 5.15; 5.10; 5.08; 4.89; 4.88; 4.55

PREPARATION 24

Methyl (1-4)-O-(benzyl 4-O-levulinyl-2,3-di-O-methyl- α -L-idopyranosyluronate)-[(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-glucopyranosyl)-(1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)],-2,3,6-tri-O-benzyl- α -D-glucopyranoside (28).

A mixture of 27 (97.3 mg; 36 μ mol) and of 25 (83.8 mg, 55 μ mol) is treated according to method 3. Column chromatography (cyclohexane-acetone 2:1, then 7:4, then 3:2) gives pure 28 (102 mg; 69%). $[\alpha]_D + 22$ ($c = 0.51$, dichloromethane). TLC, R_f 0.18, 3:2 cyclohexane-acetone.

¹H NMR (CDCl₃) δ : 5.26; 5.14; 5.10; 5.08; 4.93; 4.92; 4.90; 4.56

PREPARATION 25

Methyl (1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)-[(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-glucopyranosyl)-(1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)],-2,3,6-tri-O-benzyl- α -D-glucopyranoside (29).

The compound 28 (216 mg; 54 μ mol) is treated according to method 1 to give 29 (199 mg; 95%). TLC, R_f 0.56, 1:1

cyclohexane-acetone; R_f 0.55, 2:1 toluene-acetone. LSIMS, positive mode: m/z thioglycerol + NaCl, 3934 (M+Na)⁺; thioglycerol + KF, 3950 (M+K)⁺.

5 PREPARATION 26

Methyl (1-4)-O-(benzyl 4-O-levulinyl-2,3-di-O-methyl- α -L-idopyranosyluronate)-[(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-glucopyranosyl)-(1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)],-2,3,6-tri-O-benzyl- α -D-glucopyranoside
 10 (30).

A mixture of 25 (33 mg, 21.4 μ mol) and of 29 (52.4 mg; 13.3 μ mol) is treated according to method 2. Column chromatography (7:4 cyclohexane-acetone) gives pure 30 (43.8 mg; 62%). $[\alpha]_D + 19$ (c = 0.5, dichloromethane). TLC, R_f
 15 0.36, 3:2 cyclohexane-acetone. LSIMS, positive mode: m/z thioglycerol + KF, 5310 (M+K)⁺.

¹H NMR (CDCl₃) δ of the principal anomeric protons: 5.26; 5.14; 5.10; 5.08; 4.92; 4.90; 4.56

20 PREPARATION 27

Methyl (1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)-[(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -glucopyranosyl)-(1-4)-O-(benzyl 2,3-di-O-methyl- α -L-idopyranosyluronate)],-2,3,6-tri-O-benzyl- α -D-glucopyranoside
 25 (31).

The hexadecasaccharide 30 (100 mg; 19 μ mol) is treated according to method 1 to give 31 which is used directly in the following step. $[\alpha]_D + 20$ (c = 0.38, dichloromethane). TLC, R_f
 30 0.31, 4:3 cyclohexane-acetone. LSIMS, positive mode: m/z thioglycerol + NaCl, 5196 (M+Na)⁺; thioglycerol + KF, 5212 (M+K)⁺.

PREPARATION 28

Methyl (1-4)-O-(benzyl 4-O-levulinyl-2,3-di-O-methyl- α -L-idopyranosyluronate)-[(1-4)-O-(3,6-di-O-acetyl-2-O-benzyl- α -D-glucopyranosyl)-(1-4)-O-(benzyl
 35 2,3-di-O-methyl- α -L-

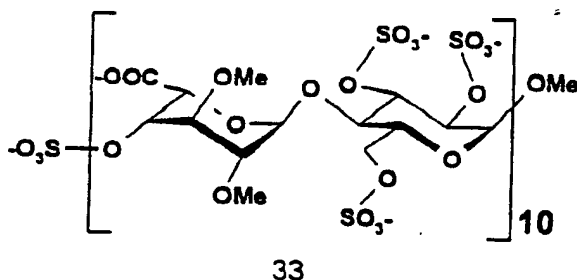
idopyranosyluronate)],-2,3,6-tri-O-benzyl- α -D-glucopyranoside (32).

A mixture of **25** (31 mg; 21.7 μ mol) and of **31** (94.5 mg, 18.3 μ mol) is treated according to method 3 to give **32** after several column chromatography steps (29.2 mg; 25%). $[\alpha]_D + 22$ ($c = 0.33$, dichloromethane). TLC, R_f 0.26; 4:3 cyclohexane-acetone. ^1H NMR (CDCl_3) δ of the principal anomeric protons: 5.26; 5.14; 5.10; 5.09; 4.92; 4.91; 4.90; 4.56 ppm.

10 EXAMPLE 1

Methyl (1-4)-O-(2,3-di-O-methyl-4-O-sulpho- α -L-ido-pyranosyluronic acid)-[(1-4)-O-(2,3,6-tri-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl- α -L-idopyranosyluronic acid)],-2,3,6-tri-O-sulpho- α -D-glucopyranoside, sodium salt

15 (33).



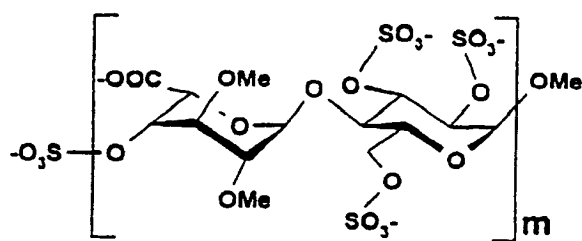
Compound **32** is treated according to method 4 to give **33** (60% over the three steps).

25 $[\alpha]_D + 27$ ($c = 0.4$, D_2O); ESIMS, negative mode: experimental mass = 7077.3 $\pm$ 3.2 a.m.u.

^1H NMR (D_2O) δ principal anomeric protons: δ 5.41; 5.40; 5.15; 5.09; 5.07; 5.06 ppm.

Proceeding according to EXAMPLE 1 and according to
30 SCHEME 1 above, compounds **34** to **38** (EXAMPLES 2 to 6) described in TABLE I below are prepared.

TABLE I



(I.1)

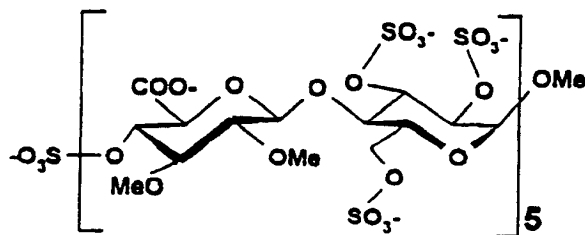
Example number	m	$[\alpha]_D$	Experimental mass
2 Compound 34	5	+30	3605
3 Compound 35	6	+29	4297
4 Compound 36	7	+27	4993
5 Compound 37	8	+34	5688
6 Compound 38	9	+27	6381

10

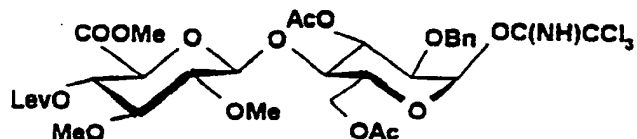
EXAMPLE 7

Methyl (1-4)-O-(2,3-di-O-methyl-4-O-sulpho-β-D-glucopyranosyluronic acid)-[(1-4)-O-(2,3,6-tri-O-sulpho-α-D-glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl-β-D-glucopyranosyluronic acid)],-2,3,6-tri-O-sulpho-α-D-glucopyranoside, sodium salt (39).

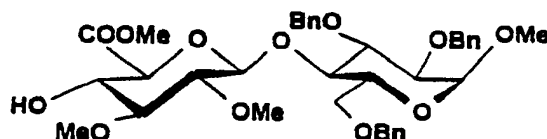
15



The glycosyl donor disaccharide



and the glycosyl acceptor disaccharide



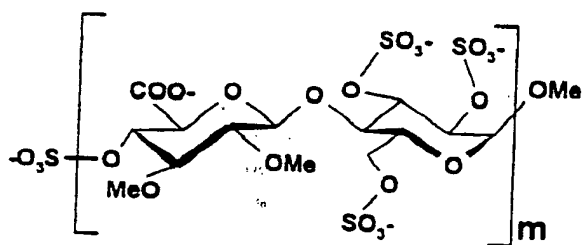
are prepared according to the methods described in Westerduin et al., BioOrg. Med. Chem., 2, 1994, 1267, then combined as described during the preparation of **34**. The resultant decasaccharide is treated according to method 4 to give **39**.

$[\alpha]_D + 45$ ($c = 1$, H_2O). ^1H NMR (D_2O) δ of the principal anomeric protons: 5.53; 5.18; 4.65; 4.63 ppm.

Proceeding according to EXAMPLE 7 and according to SCHEME 1 above, compound **40** (EXAMPLE 8) described in TABLE II below is prepared.

20

TABLE II



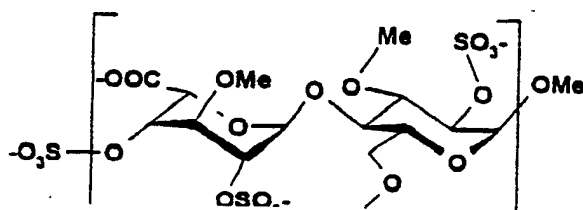
(I.1)

Example number	m	$[\alpha]_D$	^1H NMR (D_2O) δ (ppm) of the principal anomeric protons
8 Compound 40	4	+45	5.53; 5.13; 4.62; 4.60

EXAMPLE 9

Methyl (1-4)-O-(3-O-methyl-2,4-di-O-sulpho- α -L-ido-pyranosyluronic acid)-[(1-4)-O-(3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(3-O-methyl-2-O-sulpho- α -L-idopyranosyluronic acid)],-3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranoside, sodium salt (41).

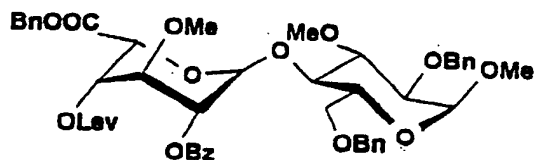
10 synthon



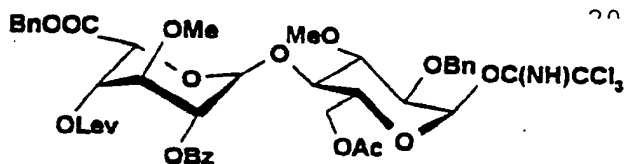
The disaccharide

(TLC, R_f

15 is treated
12 to give
imidate

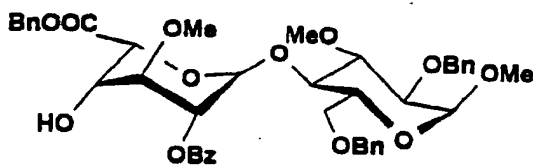


0.54, 1:1
cyclohexane/EtOAc)
as described for
the glycosyl donor



and the acceptor

25 ^1H
7.15 (m,
(d, 1H,
1H, H-1),
(3s, 3 OCH₃)).



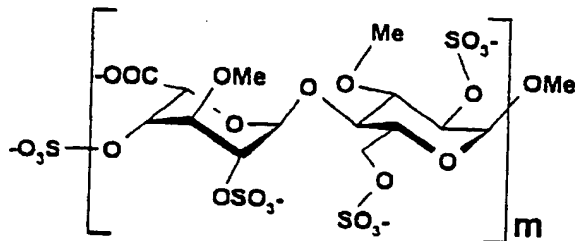
NMR (CDCl₃) δ 8.00-
20H, 4Ph), 5.15
H-1'), 4.57 (d,
3.48; 3.47; 3.32

30 These three synthons are then combined as is described
for the preparation of 34. The resultant decasaccharide is then
treated according to the method 4 to give 41.

$[\alpha]_D + 17$ ($c = 1$, H₂O). ^1H NMR (D₂O) δ of the principal
anomeric protons: 5.36; 5.34; 5.13; 5.11; 5.09; 5.05 ppm.

35 Proceeding according to EXAMPLE 9 and according to
SCHEME 1 above, the compounds 42 and 43 (EXAMPLE 10 and 11)
described in TABLE III below are prepared.

TABLE III

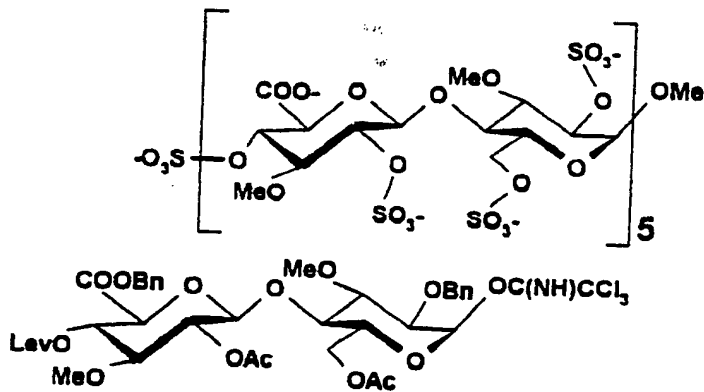


(I.2)

Example number	m	¹ H NMR (D ₂ O δ (ppm) [600 MHz] of the principal anomeric protons
10 Compound 42	4	5.20; 5.17; 5.12; 5.03; 4.90
11 Compound 43	6	5.20; 4.99; 4.95; 4.90

5 EXAMPLE 12

Methyl (1-4)-O-(3-O-methyl-2,4-di-O-sulpho-β-D-glucopyranosyluronic acid)-[(1-4)-O-(3-O-methyl-2,6-di-O-sulpho-α-D-glucopyranosyl)-(1-4)-O-(3-O-methyl-2-O-sulpho-β-D-glucopyranosyluronic acid)],-3-O-methyl-2,6-di-O-sulpho-α-D-glucopyranoside, sodium salt (44).



The disaccharide synthons

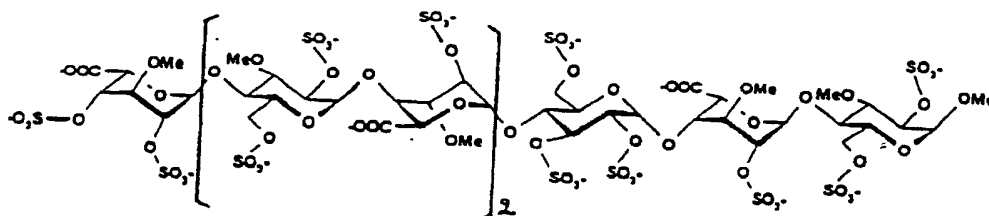
are prepared and then combined as described for 34. The decasaccharide obtained treated according to method 4 gives 44:

$[\alpha]_D + 25$ ($c = 0.2$, H_2O). ^1H NMR (D_2O) δ of the principal anomeric protons: 5.47; 5.07; 4.74; 4.71; 4.70 ppm.

5

EXAMPLE 13

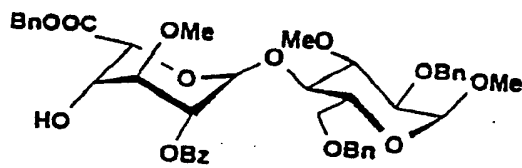
Methyl (1-4)-O-(3-O-methyl-2,4-di-O-sulpho- α -L-ido-pyranosyluronic acid)-[(1-4)-O-(-O-methyl-2,6-di-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(3-O-methyl-2-O-sulpho- α -L-ido-pyranosyluronic acid)]₂-(1-4)-O-(2,3,6-tri-O-sulpho- α -D-glucopyranosyl)-(1-4)-O-(3-O-methyl-2-O-sulpho- α -L-idopyranosyluronic acid)-3-O-methyl-2,6-di-O-sulpho- α -D-glucopyranoside, sodium salt (45).



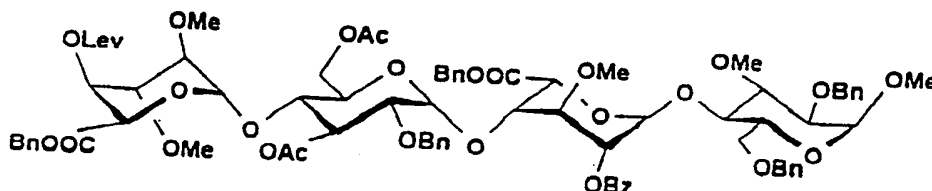
15

The glycosyl donor 15 and the glycosyl acceptor

combined
20 method 3

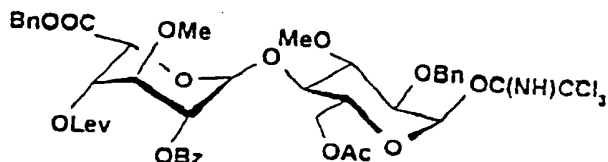


according to
give the
tetrasaccharide



After cleavage of the Lev group (method 1) and repeated
25 reaction with the following glycosyl donor disaccharide,

according to the principle described in Scheme 1,



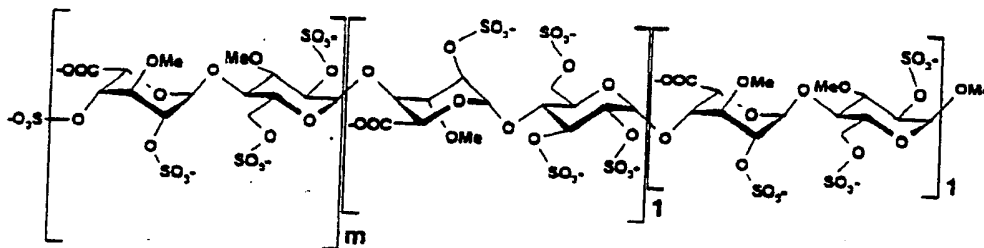
the completely protected precursor of **45** is obtained,

which is treated according to method 4 to give **45**.

¹H NMR (D₂O) δ (ppm) [600 MHz] of the principal anomeric protons: 5.35; 5.33; 5.30; 5.22; 5.21; 5.18; 5.15; 4.98.

Proceeding according to EXAMPLE 13 and according to SCHEME 1 above starting from completely protected oligosaccharide precursors, compound **46** (EXAMPLE 14) described in TABLE IV below is prepared.

TABLE IV



(I.3)

20

Example number	m	¹ H NMR (D ₂ O) δ(ppm) [600 MHz] of the principal anomeric protons
14 Compound 46	3	5.34; 5.32; 5.27; 5.22; 5.14; 4.98